AminoFormula

Goal

To supply proportionate to the demand, the primary signaling molecules, leucine, isoleucine, valine, histidine, phenylalanine, threonine, lysine and methionine that are directly responsible for triggering muscle protein synthesis (MPS) in an isolated, low calorie and powdered form allowing timely, rapid, and relatively unobstructed transport to skeletal muscles to help support a net whole body positive protein balance beyond what is normally achieved with intact proteins containing these eight amino acids. Proper dosing would establish the desired hyper-aminoacidemia environment conducive to delivering:

1) An additive MPS effect to the daily ingestion of intact protein to optimize the user's exercise induced MPS/recovery potential to maximize and prolong training gains. Fully recover and avoid training plateaus by delivering a highly anabolic formula in extreme low calories in support of controlling body composition while pursuing muscle size and performance goals.
2) To the non-exercising adult population, a low calorie MPS supplement to slow declining net muscle protein balance starting in third or fourth decade of life that leads to age-related muscle loss. Slow muscle loss and related aspects (e.g., balance, bone, strength, injury, etc.) in relatively low calorie/nitrogen/sulfur load.
3) A low nitrogen and sulfur producing anabolic triggering supplement in a flexible low calorie starting formula (add food/supplement components as desired) to support protein needs for anyone, which may be important to the rapidly expanding aging population hampered by age-related muscle loss combined with or without loss of appetite or diminishing organ functions.
4) A pre and post activity supplement for exercisers controlling calories during aggressive weight/fat loss including integration within a weight/bodyfat reduction meal replacement/substitute program.
5) The ability to control body composition while enhancing muscle size and performance gains by a means (timing of skeletal muscle hyper-aminoacidemia) incremental to other MPS mechanisms such as, but not limited to exercise, traditional pre/post exercise foods or intact protein feedings, meal timing, cell volumizing, carbohydrate, creatine or beta-alanine loading, etc.

Rationale

This document will form the rationale for the use and basis of formulation for the AminoFormula product. For those practitioners wanting detailed background information on protein and amino acids in exercise, growth and development, weight/bodyfat reduction, muscle protein synthesis (MPS), muscle protein breakdown (MPB) and overall health and aging including comprehensive information on the interplay between exercise protocols and protein intake (e.g., food/supplement sources, mechanisms of action, goal specific requirements, timing, muscle full effect, etc.), they are referred to the Protein in Exercise section (WheySmooth) in The Practitioner Dietary Supplement Reference Guide (PDSRG).

Eight (8) essential amino acids, (EAAs) leucine, isoleucine, valine, histidine, phenylalanine, threonine, lysine and methionine are the anabolic agents in protein that trigger/message the MPS process with or without exercise. While leucine is the primary activator of MPS, the other seven actuators need to be present in proper/complementary amounts or the EAA with the lowest concentration relative to the demand, will restrict the anabolic response no matter how much leucine or other amino acids (AAs) are available.
Researchers have demonstrated that consuming these eight molecules in isolation proportionate to the demand allows a greater and faster increase in skeletal muscle (SM) aminoacidemia, which stimulates more robust rates of MPS, than intact protein. This rapid increase following a balanced ingestion allows these low-calorie anabolic agents to be timed as necessary around exercise and meals to create an additive effect on

MPS to what the normal required daily intake of intact proteins produces. Further, these low-calorie anabolic agents may help achieve and control desired body composition goals while maximizing MPS.

Background

The maintenance of skeletal muscle (SM) tissue, like the mass of other tissues, is dependent on the ongoing processes of protein and cell turnover.[1,2] Therefore skeletal muscle mass is a result of the degradation (catabolism) of existing SM proteins and synthesis (anabolism) of new tissue throughout life.[2,3,4] In the normal non-exercising, well fed human from birth through puberty and, based on daily activities, sometimes into the third decade of life, SM protein balance is generally positive, meaning anabolism outpaces catabolism leading to SM growth and strength increases (positive SM protein balance).[4,5,6,7,8,9] Exercise can stimulate natural human SM synthesis and muscle performance throughout life when compared to a non-exercise state and at a minimum, attenuate the obligatory age-related muscle loss when SM protein balance tilts to negative.[4,10,11,12,13,14]

The general goal of most athletes is to maintain a positive SM protein balance and in fact maximize the body's natural MPS processes while minimizing the necessary exercise induced muscle protein breakdown (MPB)[15] to recover adequately from each training bout to ultimately enhance performance and if desired, increase SM size. In other words, the goal of athletes is to continue to improve physically by making each training session build on the previous, thus leading to continuous athletic/physical progress.[16,17,18, 19,20,21,22] Eventually, as in all aspects of life, age will become a factor in abating progress and despite exercise's constant MPS initiation or stimulus, positive training progress slows significantly with age and experience (the younger and/or less experienced, the more gains[23,24,25]),[14,26] and training plateaus become common occurrences.[17,27,28,29]

Although at some point aging clearly blunts the human response to exercise and nutrition, and eventually there will be a decline in performance,[30,31,32] this inevitable decline can be prolonged to a point where one has the greatest potential to not just extend their athletic success or "playing/competitive lifespan", but for anyone wanting to remain self-sufficient throughout life by slowing muscle loss and related aspects (e.g., balance, bone, strength, injury, etc.).[4,27,28,33,34,35] For this reason, scientists continue to search for nutrition protocols that may maximize daily MPS under all circumstances, to not only extend the life period of exercise-induced gains, but also to delay the inevitable performance decline for all humans.

Supplementing the diet with intact protein formulas (containing all 20 amino acids [AA]) such as whey (highest biological value), soy, casein, etc., and especially in close proximity to exercise, is a long-held tactic to help maximize MPS.[20,21,22,36,37] And although complete intact proteins contain all essential amino acids (EAA), recently, researchers have discovered that the MPS response from the ingestion of isolated EAA supplementation (the anabolic components of intact protein[38,39]) is greater than twice the response from a gram/gram comparable dosage of whey protein isolate.[40,41] These results carry on from previous data such as the Drummond et al. findings that resistance exercise (RE) alone produced a 40% increase in MPS above fasted levels,[42]and intake of EAAs and carbohydrate (CHO) caused a 100% increase in MPS over the same fasted baseline, while exercise with EAA supplementation (EAAS) and CHO working synergistically produced 145% increase in MPS.[42] Further, it was shown that RE alone increases muscle fiber cross sectional area (CSA), but RE supplemented with EAAs and CHO together further improved training adaptations, purportedly from the mitigation of normal post-exercise muscle protein breakdown (MPB).[43,44] Therefore, a primary mechanism of action from EAAS ingestion combined with intact protein has been surmised to be the greater suppression of MPB leading to increases in net protein balance compared to intact protein alone. Taken together, since ingestion of intact protein and isolated EAAS have been found to have unique and additive MPS results, and properties including functional absorption rates (arrival to target tissues – speed and amounts), supplementing them both in a timely fashion may yield greater long-term benefits than either protocol alone.

Exercise, Protein and EAAs to Maximize and Prolong a Positive Muscle Protein Balance Throughout a Lifetime

Intact Protein, free EAAs and exercise can, at the molecular level, stimulate skeletal muscle protein turnover independently.[41,45,46,] Properly combined, their respective mechanisms of action may function additively in favoring MPS to produce a prolonged positive net whole body protein balance (MPS>MPB) including SM as measured by whole body protein kinetics (whole body anabolic response) and muscle fractional synthetic rate (FSR) respectively.[14,15,41,45,46,] Therefore, because of differences in digestion and absorption times, and AAs splanchnic extraction (non-SM destinations), proper sequencing the ingestion of all three amino acid sources (traditional foods, intact protein powders and isolated EAAs) can allow an intracellular hyper-aminoacidemia condition that may enhance net whole body protein balance (WBPB) including MPS, or exercise induced gains beyond simply meeting protein requirements.[40,41,45,46,47,48] The different rates of AA appearance and amounts following ingestion into the intracellular space of muscle cells (inward AA flow also stimulates the inward transport of the AA) from the three exogenous sources, allows the participant control over daily timing and amounts, especially in close proximity to exercise, to achieve a AA saturation/messaging that may maximize and prolong MPS, taking full advantage of exercise-induced MPS stimulation that always takes place but may often not be fully realized because of incomplete nutrition.[18,36,38,45,49] The original research that gave rise to this type of supplementation demonstrated that the rate of protein synthesis can be favorably altered by the intracellular availability of AA (not intramuscular) and the sensing of the concentration of extracellular AA.[47] To be sure, current research demonstrating the increases in WBPB or anabolic response from combinations of EAAs and intact protein supplementation versus intact protein supplementation or traditional foods alone, credits the results to the mTOR (and AMP-activated protein kinase [AMPK]) sensing of these messenger EAAs, which includes signaling to suppress MPB, including exercise-induced MPB (albeit a necessary component of MPS).[18,41,50,51, 52,53]

Author's Original Product Rationale History Note

In 2005-2007, this author along with Dr. Alan Titchenal, researched and wrote the peer-reviewed chapter titled "Other Individual Amino Acids" captured in the book *Sports Nutrition: Fats and Proteins* edited by Dr. Judy Driskel and published by CRC Press.[54] The research we utilized to produce the chapter was from published works regarding the isolated eight of the nine EAAs, named above, and their contribution to MPS. As described, these researchers determined these molecules to be the anabolic components of protein and the only AAs involved in the signaling of MPS – i.e., the messenger molecules. From that information, we were able to create a product and commercialize it for the purpose of the goals identified in the Goal section of this document and distribute throughout our channels of professional and collegiate sports, including the over 1,500 sport/fitness and rehab facilities we support and through the National Academy of Sports Medicine (NASM).[55] The original researchers were testing the formula on the older population to help overcome natural aging anabolic (exercise and protein) resistance with respectable success. But we realized their discovery was at a minimum, a low dose branched-chain amino acid (BCAA) supplement that many intense training athletes, and especially when calorically restricted, were using regularly to support LBM and recovery (along with fast acting pre/post exercise protein/carbohydrate protocols, we also included tableted BCAAs with the pre/post shakes starting in the 1980s for bodybuilders and other strength and performance athletes). The discovery that there were five more AA involved in the MPS messaging/activation was an "Ah-Ha" moment for us because it meant that if the proper proportion is not supplied timely (intracellular appearance rate during exercise induced hyper-nutrient sensitivity), you could ingest all the BCAAs you want but without the other five EAAs present in proportion to the demand, maximum MPS may be limited to the lowest available AA of the eight necessary, if indeed there is more potential MPS "left on the table" (Plotkin et al. appears to have proven this theory correct[56]). More importantly, we recognized the ability of these isolated molecules to create a timely (in the user's control) and enhanced intracellular hyper-aminoacidemia environment thus potentially enhancing/accelerating recovery

including desired exercise-induced muscle adaptations. The empirical data we captured from our millions of users demonstrated a superior result compared to BCAAs and/or adequate protein alone including the use of pre/post exercise protein/carbohydrate shakes. We labeled the protocol "protein stacking" that included a total of 1 gram of complete/intact protein per pound of LBM daily split between meals (~every four hours), a pre-workout intact protein shake ~40 minutes before activity and the post workout shake ~ 30 minutes AFTER the last amino acid formula dose, which was consumed twice. First EAA dose starts ~10 minutes before exercise and can continue to be consumed during (generally finished at the one-quarter or halfway point), and the same dose repeated immediately post exercise. This protocol is still being used by many competitive athletes and intense exercisers, and subsequent research publications appear to validate that we may been ahead of our time. For interested readers, we published a follow up document for our practitioners in 2015 that included updated research supporting the protocol. The remainder of this update will focus on current support for use of these specialized formulas while the original formula basis can be found in the two linked references above for interested readers.

Basic Mechanism of Action

Timely, proportionate to the demand, isolated delivery of protein's anabolic agents into the mTOR1 SM signaling pathway creates a hyper-aminoacidemia environment conducive to maximizing MPS.

Introduction/Review

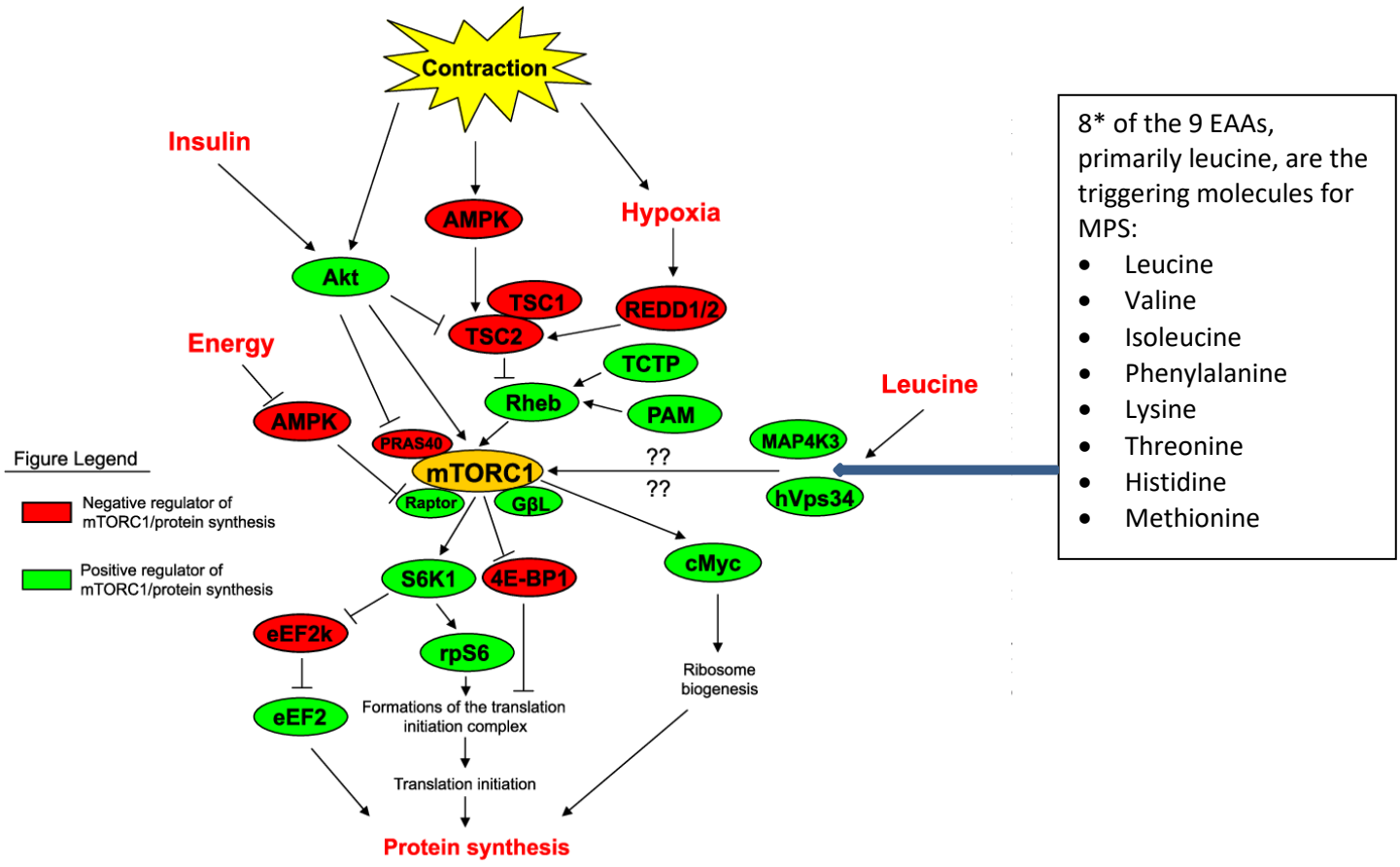
Without an endogenous source of protein and its constituent AAs, SM hypertrophy cannot take place at any stage in life.[17,18,20,57,58] Further, consuming AAs stimulates the MPS response through unique channels whether initiated by exercise or not.[18,38,47] As depicted by Drummond et al. in Figure 1, together, exercise triggers its MPS channels through mechanical loading,[17,18,20,50,51,59] while EAAs further enhance this mTORC1 activation, and other intracellular AA sensing mechanisms such as the human vacuolar protein sorting-34, (hVps34)[60,61,62,63] and also by increasing AA transporter expression.[64,65,66] Interested readers see *Protein in Exercise* for more details.

In brief terms, exercise triggers MPS through unique channel activation that includes signals from muscle protein breakdown (release of AA), which induces a heightened nutrient demand and their respective receptors sensitivity allowing exogenous AAs to be delivered timely in the right amounts (specifically EAA including a relatively high leucine content) to help maximize an individual's MPS potential.[18,27,41,45,50,51,52,53,60,61,67,68,69,70] And although non-essential amino acids (can be synthesized de novo from other substances/AA) are necessary components of complete muscle tissue,[1,2,3] they are not required to stimulate MPS.[38,61,71,72] As described throughout, the stimulation of MPS or anabolic response from AA is dependent on the EAA present in the intracellular space,[73] whether released during MPB or inward influx from plasma.[47,74] Additionally, it has been shown that EAA feedings can stimulate protein synthesis independently or incrementally to intact protein alone – i.e. EAA can have an additive effect on MPS.[18,41,52,53,75,76,77]

In summary, mTORC1 is the primary complex that determines muscular growth via sensing the cellular contents before initiating the building processes. Mechanical loading (exercise) and the presence of AA, specifically the EAA, regulate the activation of mTORC1. The outcomes of intracellular EAA are protein synthesis through charging the appropriate transfer ribonucleic acid (tRNA), oxidation/energy, or efflux back to plasma.[58,59,74] In the post-absorptive state (fasted), the major source of intracellular EAA appearance is from an accelerating rate of MPB, which is also the principal determinant of the amount of intracellular EAA available as precursors for MPS.[15,47,78] Recycling of EAAs from MPB is not 100% efficient, and of course offers no chance of increasing the SM protein pool (hypertrophy), and therefore without an exogenous source of EAAs, there will be a net loss of muscle protein.[2,3,4,14,17,18,20,47,57,58,79,80] Further, normal progressive exercise accelerates MPB under any circumstances, which heightens the nutrient demand and their respective receptors sensitivity, permitting exogenously derived EAA supplied proportionate to the demand, and timely in an energy rich environment, to maximize an individual's MPS potential. Therefore, depending on age, experience, energy supply (allotted

calories for body composition goal), timely saturation of the EAAs (anabolic molecules) in the intracellular space, may lead to enhanced size and/or performance training induced results when all else is equal (e.g. training, overall protein intake/diet, etc.) or simply act as a low calorie isolated EAA supplement in support of SM recovery and maintenance under any conditions.[22,35,36,41,52,53,54,81,82,83]

Figure 1 – The mTORC1 signaling pathway is driven by muscle contraction, insulin, essential amino acids (especially leucine) and energy supply and shows the positive and negative influencers of MPS when diet and exercise converge. Source: Drummond et al.[59]



Abbreviations: AMPK, AMP-activated protein kinase; Akt, protein kinase B; TSC1, tuberous sclerosis complex 1; TSC2, tuberous sclerosis complex 2; REDD1/2, regulated in development and DNA damage responses; Rheb, Ras-homologue enriched in brain; TCTP, translationally controlled tumor protein; PAM, protein associated with Myc; Raptor, regulatory associated protein of mTOR; G L, G protein -subunit-like protein; MAP4K3, mitogen activated protein kinase-3; hVps34, human vacuolar protein sorting-34; S6K1, p70 ribosomal S6 kinase 1; 4E-BP1, 4E binding protein 1; eEF2k, eukaryotic elongation factor 2 kinase; eEF2, eukaryotic elongation factor 2; rpS6, ribosomal protein S6; PRAS40, proline-rich Akt substrate-40.

Supporting these findings as depicted in Figure 1, Fujita et al. used a leucine enriched EAA mixture of .16 g/lb of fat free mass (FFM) containing histidine (8%), isoleucine (8%), leucine (35%), lysine (12%), methionine (3%), phenylalanine (14%), threonine (10%), and valine (10%), and demonstrated that EAA availability is the primary regulator of mTOR signaling and muscle protein synthesis as described above.[38] The study's conclusion was that EAA and glucose *inhibit* AMP-activated protein kinase, ([AMPK] plays critical roles in regulating growth and reprogramming metabolism), and activates mTOR signaling in SM leading to an increase in MPS due to enhanced translation initiation and the signaling that promotes elongation.[38,51,84,85] Further elucidating EAA mechanisms of actions, Carlin et al. used 13 g of EAA (2.4 g of leucine) and demonstrated an increase in SM Ras-related GTP Binding B (RAGB) mRNA (60%), mTOR phosphorylation (30%), leucine concentrations (130%) and

protein abundance (100%), all leading to a 50% increase in protein synthesis.[83] They concluded that EAAS ingestion increases RAGB expression, which may be an important target for maximizing protein synthesis. Moreover, Robinson et al. found that EAAS also increased skeletal muscle mitochondria protein synthesis and oxidative enzyme activity in healthy humans, suggesting an additional exercise recovery benefit.[82]

Whole-Body Protein Synthesis (WBPS) & Whole-Body Protein Balance (WBPB)

Drummond et al. demonstrated that increasing the availability of EAA independently up-regulates skeletal muscle amino acid transporter expression to help maximize inward flow.[65] EAA administered timely including before, during, or after exercise has also been shown to have an inhibitory effect on muscle breakdown,[41,44,52,53,86,87,88] which would be expected since exercise produces an increase in net amino acid efflux (proteolysis) during activity due to the loading of SM and a depression in signaling of protein synthesis[29,59,89,90] (see Figure 1). Because the intracellular appearance of EAAs from MPB and/or their inward influx from plasma, as described above, govern the anabolic response, timely influx of EAAs (supplementing isolated EAAs) may offset too many (some is necessary) negative MPS signals caused by exercise, stresses including diet, or the post-absorptive state, when compared to a non-supplemented condition, allowing a better recovery and a longer-term improved WBPB equation.[41,44,52,53,86,87,91,92] Mindful that human SM has evolved to be "triaged" – i.e. expendable as it serves as a reservoir of AAs for other tissues that support life such as cellular energy requirements including splanchnic organs during energy deficits and other stresses leading to catabolism.[93] Therefore, exogenous sources and SM EAAs can and will be redirected to support immediate survival versus long-term health (SM maintenance) or "bigger biceps,"[2,6,14,18,51,92,94,95] a condition that supplementation with properly dosed isolated EAAs may attenuate through their unique transit kinetics and their subsequent flooding of the intracellular space of muscle cells. Based on the foregoing, data will be related to the use of supplemental isolated EAAs in relation to muscle and ***whole-body protein balance*** (or net WBPB) with a focus on the anabolic response loosely described as MPB (difficult to ascertain other than final outcomes based on study results) compared to MPS. Maximizing exercise induced adaptations (e.g., hypertrophy, recovery, etc.), controlling body composition, and staving off the inevitable age related decline in muscle mass for anyone offers the rationale for EAAS in conjunction with food intake.

In summary, orally ingested proteins containing the EAAs necessary for MPS signaling and subsequent muscle synthesis come in different formats such as but not limited to, whole foods, protein powders, and isolated EAAs, all having unique systemic digestion, absorption and transport kinetics, with the latter having the greatest ability to create an on demand anabolic environment – i.e., timing the desired intracellular aminoacidemia that favors MPS, which includes suppression of MPB.[14,41,53,88,91,92] Further, since SM can be triaged as described above, and SM only accounts for 25-50% of whole-body protein turnover, a range driven by metabolic status (e.g., physical or dietary stresses),[6,51,92,93,94,95,96] and many tissues have higher turnover rates,[97,98,99] EAAS outcomes should be considered from a global protein synthesis perspective, because anything that improves WBPB compared to a non-supplemented state, may be improving SM protein balance through increases in anabolism and/or decreases in catabolism (i.e., sparing SM), especially during physical challenges.[41,91,92,95,100] ***Therefore, going forward, whole body rates of protein synthesis (and breakdown), which includes SM and other organs, will often be considered in determining net protein balance or anabolism/anabolic responses to EAAS***. Confirming this direction, was the Church et al. work investigating how changes in peripheral EAA following ingestion of different proteins and EAA formats affected muscle and whole-body protein synthesis.[91] Their data came from four recent studies that used primed, constant infusions of L-(ring-$^2H_5$)-phenylalanine and L-(3,3-$^2H_2$)-tyrosine to determine FSR of muscle protein, WBPS, and circulating EAA concentrations. They found that greater peripheral EAA concentrations are indeed related to the stimulation of MPS and WBPS and in response to feeding. The strongest predictors for postprandial (after feeding) FSR, ΔFSR, and ΔWBPS were measures of EAA concentrations, explaining ~30–50% of the variances found in protein synthesis measures. These findings support previous works demonstrating the requirement for increased peripheral EAA concentrations to

stimulate MPS and WBPS, and that the larger EAA gradient between the extracellular (peripheral) and intracellular compartments drives greater inward transport and subsequent increases in the stimulation of synthesis.[47,101] Further, the authors found, based on the average basal EAA concentrations (961 µmol/L), their compounded result indicated a 100% increase in EAA concentrations (Max EAA Concentration = 1922 µmol/L) would result in a ΔFSR of 0.020, or a ~34% increase (based on post-absorptive [4 hours fasted] FSR).
The author's conclusion was as follows: "Taken together, EAA sources that produce a large and rapid increase in peripheral EAA concentrations are recommended to improve muscle and whole-body protein synthesis."[91]

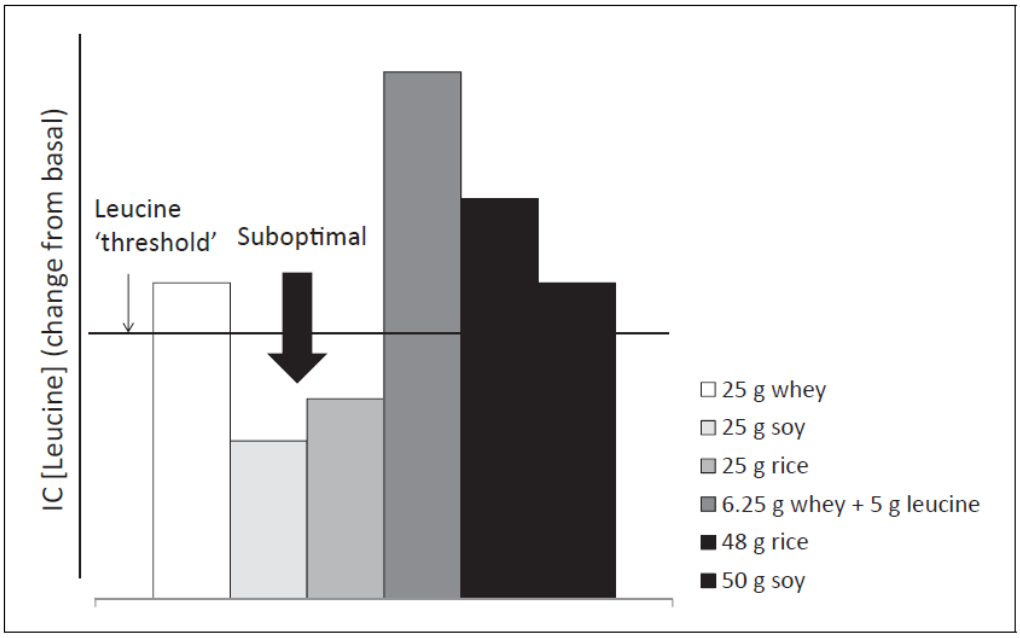
Early Research on EAA Supplementation in MPS With and Without Exercise

The discovery of the direct effects of EAAs on MPS started the avalanche of research committed to finding a way to get an exogenous source of these anabolic molecules delivered to muscle cells where they might improve muscle protein balance throughout a lifetime.[71,73,78,80] However, as cited above, the notable Bohe et al. research demonstrating that MPS was related to the inward AA transport into the extracellular space, not intramuscular (specifically EAAs concentrations),[47] set the foundation for the ongoing research combining different protein formats with isolated EAA supplementation to favorably support/control MPS under various conditions such as energy restriction, aging including anabolic resistance,[28,102,103] wound/surgical healing, weightlessness,[104,105] and general exercise recovery under all situations, to produce favorable body composition and/or performance outcomes compared to no supplementation. [18,41,44,52,53,75,76,77,86,87,89,90,91,92,93,94,96,106,107,108]
Early examples of the strength of small amounts of EAA supplementation's effects on MPS were demonstrated by Bukhari et al. and Coker et al., with the former showing only 3.0 g of EAA (40% leucine) to be as effective at stimulating MPS as 20 g of whey protein in older women after exercise.[109] Coker et al. added 6 g of EAA to a whey protein meal replacement and compared the weight loss effects of an equal amount of intact protein in a normal meal replacement.[110] They found a greater loss of adipose tissue and an increase in skeletal muscle protein fractional synthesis rate (FSR) in the EAA treated group. Recently, following their previous work related to EAA delivery formats (EAAs, intact protein, and protein containing mixed meals),[92] Gwin et al. determined combining EAAs with high quality intact proteins (everything else equal) enhanced WBPS compared to other iso-nitrogenous formats, suggesting supplementation to be an "effective strategy to offset body protein loss during the catabolic stress of energy deficits.[111]

Power and Limitations of Leucine

As previously discussed, the importance of leucine in stimulating MPS is well established,[112,113,114,115,116] and therefore researchers conceptualize a leucine threshold for maximizing MPS as shown in Figure 2. The leucine threshold ("trigger") proposes that for maximum MPS to take place following protein ingestion, the muscular intracellular leucine concentration needs to reach a given level – i.e., "the leucine threshold.[17,66,117,118] In order to maximize MPS, this leucine threshold, depending on age and activity, may be in amounts greater than 2.5 g per protein dose.[17,66,102,118,119,120] To be sure, researchers generally credit much of whey protein's superior MPS qualities to its high EAA content, especially leucine, per gram of protein.[121] However, readers should be reminded regardless of the excess in leucine, if any of the remaining necessary seven EAAs trigger molecules are shorted relative to demand, the one(s) with the lowest concentration will be the limiting factor in the anabolic response – i.e. all EAAs required for MPS need to be supplied proportionately to the demand, with leucine as the initiator,[41,118,122] leading to the ideal leucine-enriched essential amino acid supplementation (LEAAS).

Figure 2 - Leucine Intracellular Concentration from Various Protein Sources



Source: Devries and Phillips.[121] Intracellular (IC) leucine concentration following the consumption of varied doses of protein in relation to the proposed "leucine threshold." This data is gathered from young, resistance-trained subjects therefore this "leucine threshold" would increase with age and physical inactivity.[17] The leucine threshold proposes that for maximum MPS to take place following protein ingestion, the muscle intracellular leucine concentration needs to reach a given level and the amount of leucine should be >2.5 g – i.e. "the leucine threshold.[66,118,119,102]

Although LEAAS has demonstrated added value in acute signaling of MPS, the other EAAs (and NEAAs) are necessary to sustaining the process and resulting accretion throughout the day,[1,2,3, 14,17,18,123,124,125] giving rise to the use of multiple protein/EAA formats as detailed above in an attempt to maximize all MPS opportunities (and WBPS) by maintaining an anabolic friendly SM AA environment throughout the day.

Below are results of early studies involving EAAS formulas (mostly leucine heavy) that help determine successful dosing:

- Churchward et al., demonstrating the power of leucine, found by adding 5 g of leucine to 6.25 g of protein that the supplement was just as effective as 25 g of whey protein in stimulating MPS.[118]
- Rowlands et al. compared the effects of 23 g of protein spiked with 5 g of leucine to 70 g of protein spiked with 15 g of leucine and a control formula on MPS after endurance exercise.[126] Both leucine groups reached near maximal FSR, which was 33% above controls. Of note is the three times higher leucine formula produced a negligible FSR increase compared to low dose (~13%) but mTORC1 (Ser2448) phosphorylation only increased significantly in the high dose, suggesting a threshold in leucine amounts and specific MPS target activity. Serum insulin was increased in the high leucine/protein but not with the 5 g.[126]
- Dreyer et al. found that enhanced activation of the mTOR signaling pathway is partially responsible for the greater synthesis of muscle protein observed when resistance exercise is followed by EAA + carbohydrate (CHO) ingestion.[124] The formula used was a leucine enriched EAA + CHO solution consisting of histidine, 8%; isoleucine, 8%; leucine, 35%; lysine, 12%; methionine, 3%; phenylalanine, 14%; threonine, 10%; and valine, 10% at .16 g/lb (~20-25 g).[38] The leucine-enriched EAA + CHO consumed after exercise induced mTOR activation associated with a 145% increase in mixed muscle protein synthesis compared with only a 41% increase in those performing exercise alone. They concluded that the formula ingestion following a single

bout of resistance exercise significantly augments otherwise normal exercise induced MPS and may be partially explained by the increase in mTOR signaling.[124]

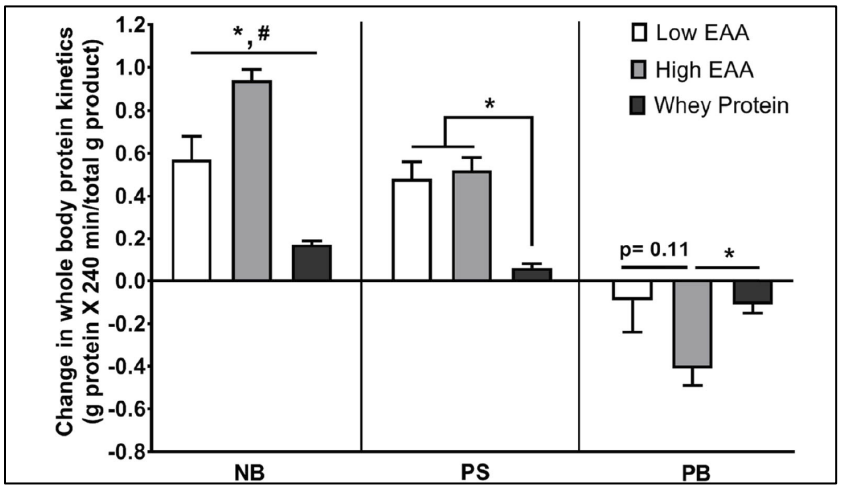
- Dickenson et al. found that ingesting a LEAA formula after resistance exercise prolongs the anabolic response and sensitivity of SM to amino acids in older adults, which may be especially important to preserving aging muscle and establishing health preserving nutrition programs.[28]
- Xu ZR et al., in a systematic review and meta-analysis through 2013, determined leucine supplementation to be effective at increasing MPS, FSR, and LBM and thus deemed it to be a potential aid for addressing age-related muscle loss.[102]
- Komar et al. found leucine supplementation to elicit beneficial effects on body weight, body mass index, and lean body mass in older persons.[127]
- Mobley et al. found that leucine, β-hydroxy-β-methylbutyrate (HMB) and creatine supplementation may independently reduce myostatin-induced muscle fiber atrophy by influencing Akirin-1/Mighty mRNA expression patterns, which may be very important to maximizing muscle hypertrophy, or at a minimum, reducing normal muscle losses.[128]
- Luiking et al. demonstrated that a low calorie leucine enriched (3 g per serving compared to 2 g in casein) whey protein source raised serum levels of total AA and EAA, including leucine, to a greater and faster extent than casein protein.[129] Based on the fact that MPS responds to extracellular EAA concentrations,[47] subsequent transport and intracellular AA rate of deposition,[58,130] and higher postprandial levels of EAA and leucine correlate to a greater protein synthesis rates, the authors suggest that the rapid postprandial profile of AA appearance in blood is especially important in older adults.[129,131] This study also supports the additional delivery of an EAAS without the presence of a caloric dense meal.
- Dreyer et al., using 20 g of EAA twice daily between meals for one week before and two weeks after total knee arthroplasty (TKA) showed three times less atrophy in the treated group versus placebo and an accelerated return to functional mobility.[132]
- Aquilani R et al. analyzed three studies using EAAS in subjects with chronic heart failure (CHF) and/or chronic obstructive pulmonary disease (COPD) and physical capacity.[33] The three studies consistently demonstrated CHF and COPD subjects improved exercise intolerance after one to three months of 8 g/day of EAA supplementation. In CHF subjects, exercise capacity increased 18.7% to 23% (watts; bicycle test), and 12% to 22% (meters) in a 6-minute walking test. Additionally, patients reduced their resting plasma lactate levels 25% and improved tissue insulin sensitivity by 16%. Results were similar in the COPD group. The authors attributed the benefits to the EAA's known effects on improving exercise intolerance through increases in muscle aerobic metabolism, mass and function (EAAS increases myofibrils and mitochondria genesis in SM), and improvement of tissue insulin sensitivity (improved glucose control).[33]

Current Studies Confirming Successful Dosing Protocols and Beneficiaries

The study by Park et al. describes and demonstrated how the use of low dose EAA supplementation can deliver an additive MPS/WBPS effect (anabolic response) to intact protein in healthy young adults.[41] They compared the whole-body anabolic response in three iso-gram treatments: intact whey protein supplementation (WPS) alone, and WPS combined with 6 g and 12 g of EAAs, measuring whole-body protein kinetics. They found a dose-dependent greater anabolic response with the addition of EAAs. The increase in net balance between whole-body protein synthesis and breakdown was greatest in the high-dose EAA/WPS subjects. The greater anabolic response was due to greater increases in whole-body protein synthesis (three-fold anabolic response increase in the 6 g group and six-fold in 12 g subjects) and a markedly greater suppression of whole-body protein breakdown (see Figure 3). Further, as shown in Figure 4, in the high dose group, the authors showed the muscle protein FSR reflected the changes in whole-body protein synthesis, also documenting a significant increase in the muscle FSR in a dose dependent manner.[41] As **Figure 5** depicts, their results also support how the faster and peak rise in EAA muscle concentrations from isolated EAA supplementation is likely the mechanism of action

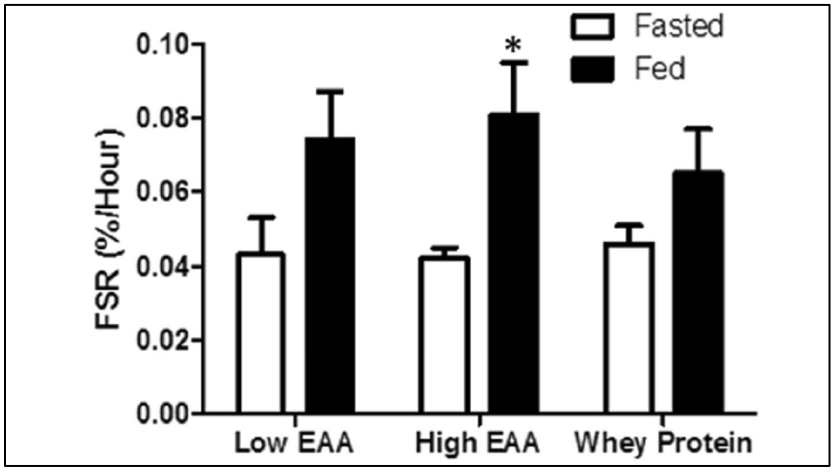
that can push protein's anabolic limits beyond the common "muscle full" FSR measurements. In other words, compared to other protein/AA formats, due to the supplemented isolated EAAs ability to quickly deliver a greater hyper-aminoacidemia SM environment (including leucine peaks), the user could timely (e.g., proximity to exercise, during energy deficits, etc.) support greater anabolism while simultaneously enhancing the suppression of MPB. To be sure, the high dose did deliver a measurable increase in both net protein balance and FSR, thus may have been partially due to the greater suppression of MPB.

Figure 3 - Net Protein Gain from EAA Combined with WPS and WPS Alone[41]



Source: Park et al.[41] Changes from baseline of whole-body net protein balance (NB), protein synthesis (PS) and protein breakdown (PB) following ingestion of the free EAAs/WPS composition (6.3 g and 12.6 g) and the whey protein product (17 g). Values are normalized for the amount of product consumed. *Statistically different from High EAA; #Statistically different between Low EAA and whey protein.

Figure 4 - Changes in Fractional Synthesis Rate (FSR) from EAA Combined with WPS and WPS Alone



Source: Park et al.[41] Muscle protein fractional synthesis rate (FSR) following consumption of one of two doses of the free EAAs/protein composition (6.3 g and 12.6 g) and the whey protein product (17.9 g). *Statistically significant from fasted within treatment.

Figure 5 below captures the faster increase and higher peak in SM EAA concentrations. Intact protein alone supplied gram per gram to EAA treatments, did not accomplish the speed or peaks[41,48,91]

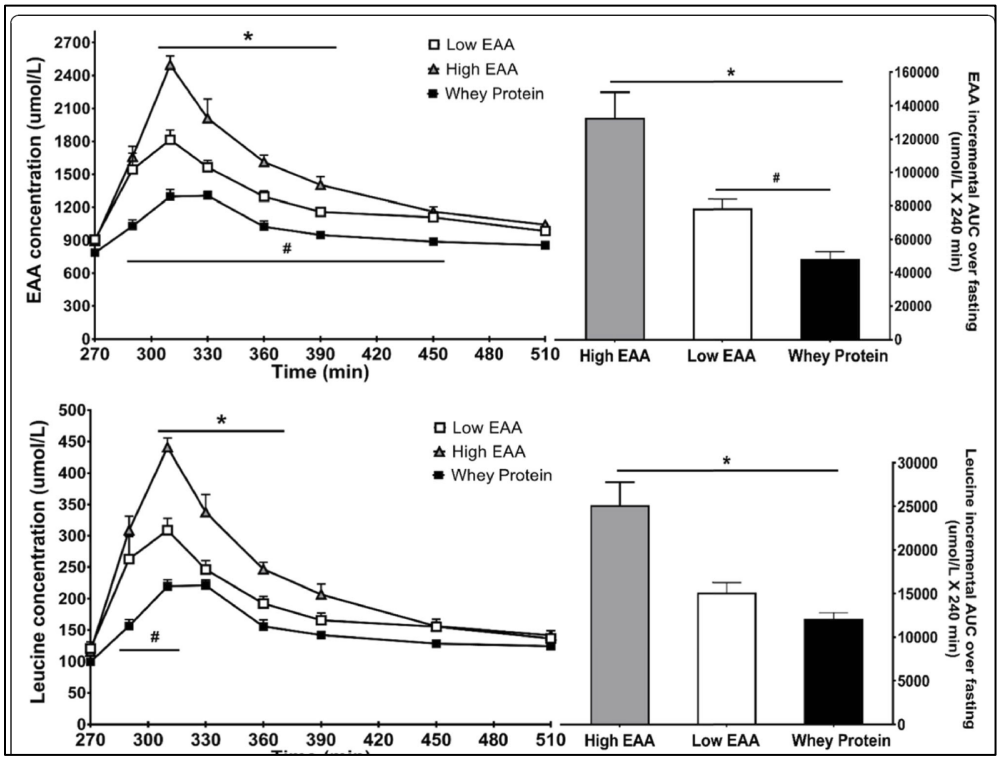


Figure 5. Total plasma EAA concentration (upper panel) and leucine (lower panel) before and following one of two doses (6.3 g and 12.6 g) of free EAA/protein composition or the whey protein alone. Bar graphs on right represent the AUC for the response above baseline over the four hours following consumption of each dose of free EAAs/protein and of whey protein. Source: Park et al.[41]

Authors conclusion: "there is an interactive effect between free EAAs and whey protein that makes their combination highly anabolic in a dose dependent manner that exceeds the anabolic response to a whey-protein based supplement by approximately three and six-fold for the low- and high-doses of free EAA/protein, respectively, when evaluated on a gram per gram basis."[41]

Using 4 g of EAAs (1.6 g of leucine) three times daily, Ford et al. found a moderate suppression of muscle damage following resistance exercise without concomitant increases in myofibrillar protein synthesis (MyoPS). The suggested recovery benefit may pay off long-term. This study was completed after four days of exercise.[133] In support of EAAS at the same dosage for recovery, Matsui et al. measured changes in serum creatine phosphokinase (CPK) activity and myoglobin concentrations as markers of muscle damage, and concluded the dosage to be effective at significantly reducing exercise-induced muscle damage in young exercisers.[88] Negro et al. administered an EAA mix at a dose of .07 g/lb, (~10-14 g) and investigated the acute effects of the mixture on myoelectric descriptors of fatigue and maximal force production after a resistance exercise protocol in young adult males.[134] The results compared to placebo, demonstrated the efficacy of pre-workout EAA mix on immediate post-resistance exercise decline of maximal voluntary contractions (MVC), endurance capacity and peripheral muscle fatigue as evaluated with multichannel surface electromyography (sEMG) myoelectric

descriptors. The conclusion suggests that EAAS, as described throughout, taken before activity/sport events or training sessions is effective in preventing early muscle fatigue and to delay task time to failure.[134]
Hirsch et al. compared the independent and combined effects of high-intensity interval training (HIIT) and EAAS on lean mass, muscle characteristics of the quadriceps, and 24-hr whole-body protein turnover (WBPT) in younger overweight and obese adults with a dosage of 3.6 g, twice daily.[135] One dose was taken before and one dose after the workout or the two doses taken between meals on non-workout days. The results were like other exercise and EAAS trials, in that they found that HIIT with EAAS may deliver greater long-term muscular adaptions (lean mass size and muscle quality) from the ability of EAAS to support WBPT including MPS and MPB. Authors also found that EAAS may be especially important to women's exercise recovery.[135]

Weight/Body Fat Loss and Energy Deficits

As discussed throughout, based on the mechanisms of action of EAAS at or above muscle saturation levels (maximum MPS levels) including suppression of MPB, supplementation may be especially helpful in supporting LBM or avoiding the triage effect (described above) during caloric restriction because EAAS improves whole-body net protein balance beyond MPS.[53,92,111,136]
Gwin et al. studied the effects of standard (0.045 g/lb) and high (0.136 g/lb) doses of free-form EAAs on exercise-stimulated mixed MPS and whole-body protein turnover during a five day 30% energy deficit.[53] As assumed, the whole-body net protein balance (NET) was greater for high compared to standard EAAS. The improvement in NET was due to greater increases in WBPS and suppression of whole-body protein breakdown in the high EAA dose compared to the standard EAA dose (insulin did not differ between treatments). But the greater increases in circulating EAA concentrations from the high dose did not increase the post exercise mixed MPS more than the standard dose, suggesting in at least the short-term, that whole body EAA requirements (e.g., splanchnic needs/survival actions) during an energy deficit take precedent over skeletal MPS. However, the high dose effect on NET supports the concept of usage during restricted eating to help offset whole body protein losses from the obligatory catabolic actions of dieting with or without exercise.[2,6,14,15,18,51,92,93,94,95] Their results also suggest a MPS saturation point during an energy deficit since the low and high dose had an equal impact on that measure but instead the effects of the higher EAAS are directed to the improvement of NET at the whole-body level. And again, this would offer value over time during restricted eating since the greater NET from the higher dose of EAAS is partially attributed to a lesser reliance on protein breakdown derived precursor AAs to support the protein synthesis in important non-muscle tissues.[15] Additionally, this study and others support evidence demonstrating that EAAs inhibit protein breakdown in a dose-dependent manner in the splanchnic area independent of insulin effects.[137] And finally, increased NET due to greater EAA availability may also reduce the proportion of ingested amino acids directed towards energy production.[15,51,53,93,94,95] Likewise, Coker et al. demonstrated that an EAA (17 g) fortified meal replacement promoted greater increments in net protein balance when compared to a standard intact whey high protein (27g) meal replacement during weight loss.[136]

Aging

For more on aging and protein, including age-related anabolic resistance, see Protein in Exercise pages 18-21.
Once the eight EAA were discovered to be the primary anabolic agents of protein,[38,71,80] EAAS began being tested in the aging population to help stave off the inevitable loss of skeletal muscle that eventually leads to adverse consequences such as loss of strength, mobility, frailty, fractures, falls, and finally the aging human often becomes a burdensome dependence until the end of days.[4,13] The rationale for supplementation in aging is predicated on the same mechanisms listed throughout: the ability to timely control a hyper-aminoacidemia environment to stimulate MPS beyond normal and adequate protein intake, or simply support intact protein intake when it is low. Mindful that ~31-50% of adults over age 51 are below the protein RDA (.36 g/lb),[138] which is significantly lower than newer older adult recommendations (0.5 to 0.8 g/lb) to help overcome the age-related anabolic resistance to the effects of protein and exercise. Therefore, by supplying the direct anabolic

agents of complete proteins, additional benefits of EAAS for the aging may be realized, such as satisfying the now established higher protein requirements[139,140,141,142,143,144,145,146,147,148,149] with less nitrogen and sulfur production, which may be important to persons with or without loss of appetite or diminishing organ functions.[150,151] Further, in exercisers, Moro et al. [152] found that EAAS effects on MPS are not hampered by normal age-related protein/exercise anabolic resistance in older healthy adults, suggesting a response independent of resistance training that may be helpful in supporting muscle mass for anyone.[150,152,153]
Negro et al. studied 12 weeks of 10 g/day (two doses of 5 g; first dose before lunch and second before dinner) of EAAS (combined with creatine and vitamin D) with no exercise intervention versus placebo in healthy older adults (average age 68 yrs). The authors demonstrated that the EAAS mixture compared to placebo positively affected muscle mass, muscle strength, muscle power and visceral adipose tissue to the point of counterbalancing more than one year of age-related loss of muscle mass and strength.[154] Other trials using multi-ingredient supplements including AA/protein and no exercise intervention, have shown similar results in protecting the loss of skeletal muscle.[153,155,156] In the same vein, Lee et al. used 7.5 g of EAAS (40% or 3 g of leucine) twice daily (15 g total) and demonstrated a robust aminoacidemia and anabolic signaling in the mTOR1 pathway (responsible for RNA translation) response compared to placebo in younger and older individuals with or without exercise.[150] Yoshimura et al. used only 3 g daily taken within 30 minutes after mild exercise in post-stroke patients and found eight weeks of the EAAS (40% leucine or 1.2 g) to significantly increase muscle mass, strength and physical function compared to placebo.[157]
Healthy older adults may prefer aerobic exercise (AE) to help maintain fitness or independence. Markoski et al. studied whether chronic EAAS or AE training (3 days per week), or a combination of the two interventions could improve muscle mass and function by stimulating MPS.[158] Participants supplemented one dose of EAA at 15 g daily (between meals on non-exercise days and in the non-exercise group, and within one hour after exercise). Both groups receiving the EAAS had acute increases in MPS but the AE and EAAS group demonstrated a chronic increase in strength. The authors noted that these were independent healthy older well-fed adults, including adequate protein intakes, suggesting a long-term benefit for any older persons. The conclusion: "In non-frail, independent, healthy older adults, AE training increased walking speed and aerobic fitness, and, when combined with EAA supplementation, it also increased muscle strength, and EAAS stimulated muscle protein synthesis." These results support the use of EAAS in well fed healthy older adults to improve muscular adaptions to exercise and increase MPS under any condition including without exercise, to support the ultimate goal of prolonging the ability to participate in desired activities and remaining independent.[158]
Beyond MPS in aging, Suzuki et al. found that ingestion of 6 g (not 3 g) of EAAS resulted in improved attention, cognitive flexibility, and psychosocial functioning.[159] While the EAAs mechanisms were unclear, the formula was designed to directly match the EAA ratios associated with the brain influx rate and support the requirement for each amino acid to maintain brain homeostasis, which naturally declines in aging, whether from diet or compromised age-related nutrient extraction.[138,159,160,161]

Dosing

Control an on-demand intracellular aminoacidemia that favors MPS based on diet, age, size and activities.

The goal for all humans (athletes, exercisers or not), would be to maintain a net positive protein balance (or stave off, minimize or attenuate any negative protein balance age-related or otherwise) as long as possible to maintain physical/performance improvements or desired active lifestyles into advancing years by delaying age-related loss of performance and function, or at a minimum, remain self-sufficient until end of days. Therefore, the aim of supplementation is to keep the MPS signaling/activity as strong as possible throughout the day by exploiting the EAA MPS pathways to maximize exercise induced tissue adaptations and overall WBPB for anyone. Proper dosing would present optimal amounts of EAA at time periods where the body could utilize them to complement other protein food forms to maximize ***whole-body protein synthesis*** (or net whole body

protein balance) including MPS such as before and after exercise or between meals throughout the day based on the individual (e.g., size, age, etc.) and their total activities.
In other words, ingestion of isolated leucine enriched EAAS (LEAAS) would support the necessary *inward* EAA transport into the extracellular space to maintain an intracellular level/concentration of EAA that keeps MPS maximized to whatever one's available daily potential may be, while minimizing MPB beyond what normal adequate intact protein feedings can accomplish. Because of multiple extenuating individual factors, there is no known exact recipe that would accomplish this for each human but based on all current data captured in this document, there is ample information to come very close with a wide range of safety.

Composition

EAAs proportionate to their demand in triggering and maximizing WBPS/MPS.

The general composition of the formulas used with success cited throughout this paper, were designed to supply the EAAs proportionate to the demand necessary to not just trigger an acute MPS response but also maximize WBPS and especially skeletal muscle synthesis, so that there is no AA limiting factor,[41,44,52,53,54,75,76,77,86,87,90,91,92,93,94,95,96,106,107,108,162] with leucine leading the way (up to 40%) based on its proposed priming and trigger effect described above.[17,42,66,102,116,117,118,119,120]

Amounts

Size, activities, diet, age, along with sport, fitness and body composition goals.

Early studies set the stage for today's formulas used in the recent studies cited above by establishing a basic dose dependency up to the "muscle full" amount - i.e. more did not increase MPS (mindful we are proposing supporting WBPB as described above) in that specific measured time period.[72,162,163] Borsheim et al. found no additional increase in MPS by increasing the EAA dose beyond 21 g (under these study conditions).[162] Tipton et al. observed equal MPS in response to the intake of 40 g of AAs composed of 18 g of EAA and 22 g of non-essential AAs, compared to 40 g of all EAAs.[72] In addition, the Borsheim study[162] neatly demonstrated that EAA ingestion following exercise stimulates MPS independently of all other mechanisms, (calories, insulin, exercise, etc.) and the authors calculated that approximately 26 g/d of muscle tissue (including muscle water content) was synthesized in response to the EAA supplementation. Two other long-term trials involving elite athletes using 6.6 g of EAA three times daily demonstrated significant improvements in physical condition and reduction in exercise induced muscle damage when compared to 2.2 and 4.4 g three times daily with everything else equal.[164,165] Because EAA are the only AA needed for MPS, you can extrapolate the same dosages from successful studies using timed ingestion of complete protein. Therefore, these early results [38,71,162,163,166,167] and those of more recent studies described above (including measuring WBPB/WBPS) that generally have higher proportions of leucine (~30-40% of total EAA content), show that the amount of EAA supplementation that might be required to maximize MPS throughout the day, (at least for the average-sized human), seems to lie between 6 and 18 g in one dose and ingested possibly 1-3 times daily depending on diet (energy or food restrictions) age (anabolic response/resistance), activities (sedentary to vigorous daily training) and/or goals (body composition, performance or remaining independent)
[41,44,52,53,54,75,76,77,86,87,90,91,92,93,94,95,96,106,107,108,150,151,152,153,154,155,156,157,158,159,160]

Timing

Purpose: diet/protein fortification, exercise recovery, aging, and/or maximize MPS for performance gains.

The timing of EAA supplementation would be situational and population based, therefore assessing all studies cited here under different situations and goals, the following conclusions can be made:

Athletes/exercisers in energy balance (or minimal caloric restriction) to prolong training-induced performance gains (i.e., maximizing MPS and controlling MPB)

- "Protein Stacking" includes use of all protein formats to induce aminoacidemia conditions that would allow an individual to take advantage of their full daily MPS/WBPS potential. Participants would consume a total of 1 g of protein per pound of LBM daily split between meals (~every four hours), a pre-workout intact protein and carbohydrate (CHO) shake ~40 minutes before activity and the post workout shake ~ 30 minutes post exercise (30 minutes after the last EAA dose). First EAA dose starts ~10 minutes before exercise and can continue to be consumed during (generally finished at the one-quarter or halfway point), and the dose repeated immediately post exercise. Hedging all bets, consume a dose before bedtime but only if not using an intact protein shake before sleep.

Athletes/exercisers supporting LBM and recovery during moderate to aggressive energy restriction.

- Same as above but may remove the pre and post workout intact protein/CHO shake to save calories.

Non-exercisers of any age as a low-calorie, high anabolic protein fortification supplement.

- Take between meals one to three times daily (range depending on daily protein intake)

Anyone seeking a low calorie, highly anabolic, but low nitrogen and sulfur producing supplement, to fortify daily protein intake because of diet restrictions, appetite loss, inactivity, aging organs, etc., to shore up protein needs and support muscle mass, particularly with aging.

- Use before and after exercise or between meals if not exercising.

Summary of AminoFormula Dose and Timing Which Suggest Benefits

Exercisers/athletes in energy balance or no more than a 20% deficit

Dose (assuming adequate protein – see above)

- For those weighing ≤ 150 lbs use 12 g per dose. One before training and repeat immediately post activity.
- Persons weighing more than 150 lbs, add 0.6 g for each additional 10 lbs of body weight (~5% for each 10 lbs of weight)
    - Example: 200 lb person would add 3 g (15 g per dose); 250 lb person would use 18 g per dose
- Aging (>50 years old) may increase dosage needs based on the body's resistance to the anabolic effects of exercise, amino acids, insulin and other related protein synthesis mechanisms in advancing years. Therefore, aging exercisers might add ~20% for each decade to their standard body weight dose shown above.
    - Example: 60 yrs at 160 lb=~15 g dose or ~1 and 1/4 scoop (12.6gm + 20% =15.12 g)

Timing (see "protein stacking" above for intact protein integration for maximizing MPS)

- Approximately 10 minutes before workout, begin ingestion and continue to consume during exercise while aiming to finish before the halfway point of the session.
- Consume same dose immediately post workout.
- Repeat dose before bedtime to potentially maximize results if not using intact protein formula before sleep.

Non-Exercisers 30 Years and Older

Dose and Timing

- 12 g per dose; (1 scoop) take three times daily between meals with one of the three doses before bedtime.

- Aging (> 50 years old) may increase dosage needs by 20% for each decade (ex: at age 60 years dosage may be ~15 g) based on the body's resistance to the anabolic effects of amino acids.

Food Form Protein Intake Fortification – Anyone

AminoFormula is purposely not a complete protein for all the reasons discussed here and therefore does not publish an amount of protein that would be officially counted as such into a person's total daily protein intake. The eight EAAs in this product are the anabolic agents within a complete protein directly responsible for protein synthesis ***activation*** and therefore serves as a low calorie, fast acting, high anabolic signaling product for activating muscle protein synthesis (MPS) beyond intact protein itself. A person's total intact protein food sources such as beef, chicken, soy, dairy including protein bars or shakes, etc., consumed throughout the day supply the remaining amino acids (also from de-novo synthesis) necessary for the complete and prolonged synthesis. Nevertheless, because it takes ~25-40 g of a complete protein (range depending on the biological value of the source) to get the amount of the eight EAA found in one serving (12 g) of AminoFormula including the 6g of BCAA, it would be conservative to add the 25 g number once a day to your protein intake as part of your 1g of protein per pound of LBM per day needs. However, active strength/performance athletes using AminoFormula regularly, still maintain the 1 g/lb/LBM/d of whole protein sources because whole protein has other helpful properties including satiety. Additionally, you could add approximately half a scoop of AminoFormula to your favorite fluid when consuming a low protein meal such as a cereal or bagel-type breakfast (e.g., continental) or other low quality or quantity protein meals, to raise the limited EAA content of the foods so that the meal has all the AA necessary for adequate MPS.

Data Summary

Creating a perfect anabolic and recovery environment through controlling SM aminoacidemia

The goal for all humans (athletes, exercisers or not), would be to maintain a net positive whole body protein balance (WBPB) as long as possible to maintain physical/performance improvements or desired active lifestyles into advancing years including delaying age-related loss of muscle function, or at a minimum, remaining self-sufficient until end of days. Therefore, the aim of LEAAS is to keep the MPS signaling/activity as strong as possible throughout the day by exploiting the LEAA MPS pathways to maximize exercise induced tissue adaptations and overall WBPB for anyone. Although intact proteins contain the EAA, researchers have discovered that the MPS response from the ingestion of specific isolated EAA (eight of the nine, i.e., the anabolic components of intact protein) supplementation is greater than twice the response from a gram per gram comparable dosage of whey protein isolate (highest BV protein source). Further, since ingestion of intact protein and isolated EAA supplementation have been found to have unique muscle protein synthesis results and properties including functional absorption rates (arrival to target tissues – speed and amounts), supplementing them both in a timely fashion may yield greater long-term WBPB including MPS benefits than either protocol alone. mTORC1 is the primary complex that determines muscular growth via sensing the cellular contents before initiating the building processes. Mechanical loading (exercise) and the presence of AA, specifically the EAA, regulate the activation of mTORC1 independently and synergistically. The outcomes of intracellular EAA are protein synthesis through charging the appropriate transfer ribonucleic acid (tRNA), oxidation/energy, or efflux back to plasma. The ingestion of leucine enriched EAA supplementation and its effect on MPS can be amplified throughout the day by supplementing protocols that maintain a specific rate and frequency of LEAA appearance in the extracellular space thus maximizing intracellular transport at given opportune MPS times. Moreover, normal progressive exercise accelerates MPB under any circumstance, which heightens the nutrient demand and their respective receptors sensitivity, permitting exogenously derived EAA supplied proportionate to the demand, and timely in an energy rich environment, to maximize an individual's MPS potential. Therefore, depending on age, experience, energy supply (allotted calories for body composition goal), timely saturation of

the EAAs (anabolic molecules) in the intracellular space, may lead to enhanced size and/or performance training induced results when all else is equal (e.g., training, overall protein intake/diet, etc.), or simply act as a low calorie isolated EAA supplement in support of skeletal muscle recovery/adaptations and maintenance under any conditions. An effective dose (i.e., proportionately supplied based on demand) appears to be 6-21 g of EAA with 3-5 g of leucine. The range is probably a function of age, body size, energy intake, exercise type, volume, and intensity, all which may drive a different usage threshold. Two to three doses consumed before and after exercise, or between meals when not exercising, may keep the MPS signaling maximized throughout the day. ***In summary, by controlling skeletal muscle aminoacidemia, AminoFormula can maximize daily activity recovery, including exercise-induced adaptations additive to intact proteins, by supplying the components (eight essential amino acids including high in the BCAAs) directly responsible for activating MPS (i.e., the isolated anabolic agents of intact protein) so that every workout builds on the previous to deliver continuous gains, avoid plateaus and support healthy aging for anyone. Because the formula is highly anabolic within extreme low calories, regular use could act as a positive influence on the regulation of muscle mass, overall health and weight control across the lifespan to continue to allow a desired body composition and activities.***

Formula Components in a 12 g Dose (1-scoop)

The EAAs, except for leucine and to a much lesser extent lysine,[168] have been rarely studied individually as ergogenic aids but generally in groups as referenced in this document and contained in the formula. As described, this formula's composition has been structured from available research to be roughly proportionate the demand for each of the eight EAAs to timely maximize MPS. In other words, when properly dosed by weight, the ratio (individual percentages) of the supplemental AAs is designed to eliminate any of the molecules from being a limiting factor in the anabolic response and subsequent favorable remodeling.
For more information on all amino acids, please see the comprehensive review by Neal Spruce and Dr. Alan Titchenal in the publication Sports Nutrition, Fats and Proteins[54]

BCAA (See above and dotFIT Recover&Build™)

- ***Leucine (4,000 mg)***
- ***Isoleucine (900 mg)***
- ***Valine (1,100 mg)***

Phenylalanine (1,670.5 mg): Phenylalanine (P) is an EAA that participates in protein synthesis.[1,2] It can be converted to tyrosine via hydroxylation. Phenylalanine is both glucogenic and ketogenic.[169] Sports drinks that contain a mixture of carbohydrate and free-form amino acids, including phenylalanine, can result in a greater insulin response than carbohydrate by itself.[170,171] P is a tyrosine precursor, and is a substrate for tyrosine hydroxylase, the enzyme that catalyzes the rate-limiting step in catecholamine synthesis.[172] The current DRI recommendation for total aromatic amino acids (phenylalanine and tyrosine) for adults aged 19 years and older is 27 mg/kg/·d.[173]

Lysine (1669.5 mg): L-lysine is an indispensable dibasic amino acid (L-2,6-diaminohexanoic acid) required for human growth and for maintaining nitrogen balance in adults.[1,2] Lysine cannot be synthesized by the body, and therefore must be supplied through diet.[174] Lysine, like most other amino acids, is a building block of body proteins. [1,2] Among the indispensable AA, lysine is present in the greatest amounts, at 93.0 mmol/dl and 38 mmol/dl in tissues and serum respectively.[168] Lysine is also required for collagen synthesis, and may be central to bone health.[175,176,177] Recent intake recommendations to meet the lysine requirement range from 64 to 30

mg/kg/d for infants (>6 months) and adults (>18 years), respectively.[173] Lysine intake in the Western human diet is in the range 40–180 mg/kg/d). An upper limit of 300–400 mg/kg/d) can be considered in humans.[178]

Threonine (1300 mg): Threonine is an EAA often low in vegetarian diets. Aminotransferases exist for all amino acids except threonine and lysine. Its main routes of catabolism lead to both ketogenic and glucogenic metabolites.[179] The human requirement for threonine set by FAO/WHO/UNU at 7 mg/kg/day[180] has been challenged by more recent data suggesting a level more than twice this amount to maintain AA homeostasis[181,182] in healthy adults. The Institute of Medicine more recently established a threonine RDA for adults at 27 mg/kg/day.[173]

Histidine (900 mg): The end product of histidine catabolism is glutamate, making histidine one of the glucogenic amino acids. Kriengsinyos et al. investigated histidine's essentiality in healthy adult humans consuming a histidine-free diet for 48 days. They discovered a gradual decrease in protein turnover and a substantial decrease in plasma protein concentrations, including albumin, hemoglobin, and transferrin. Although histidine deficiency may not affect nitrogen equilibrium, it can impact other important health parameters.[183] Histidine, like cysteine, also may have antioxidant properties.[184] In regard to sport/fitness applications, histidine alone has not been studied as a supplement for improving athletic outcomes. Carnosine is related metabolically to histidine and histamine. It is a naturally occurring histidine-containing dipeptide present in muscle tissue. Being immuno-protective, carnosine has been shown to detoxify free-radical species, protect cell membranes, and act as a buffer against lactic acid and hydrogen ions.[185] This is especially important in athletic events where lactic acid buildup (metabolic acidosis) can affect performance by causing fatigue.[21,186,187] Intracellular buffering agents such as phosphates and histidine-containing peptides may help delay fatigue by buffering hydrogen ions, reducing oxidative damage, and maintaining cell membrane integrity.[188,189,190] The daily recommended amount for histidine is 8 to 12 mg/kg of body weight per day in adults. [173,191]

Methionine (360 mg): Methionine is a major source of sulfur in human diets, and is an EAA for normal growth and development.[1,2] It is considered glucogenic, due to its conversion to pyruvic acid via succinyl CoA. It is a major methyl-donor, and important in the metabolism of phospholipids. It is also prominent in methylation reactions, and as a precursor for cysteine, which is the rate-limiting AA for glutathione synthesis. High levels of methionine are associated with hyperhomocysteinemia and endothelial dysfunction, which are risk factors for cardiovascular disease.[192] Deficiency of methionine produces hepatic steatosis similar to that seen with ethanol use,[193] and supplementation with this lipotrope can prevent ethanol-induced fatty liver.[193] Besides methionine's role in methyl-group metabolism, and in serving as a substrate for protein synthesis, its other functions include participation in the synthesis of polyamines, catecholamines, nucleic acids, carnitine, and creatine.[194,195,196] Because of its many functions, methionine has a high intracellular turnover.[197,198] It may be the amino acid that is most rate-limiting for the building of body proteins, including maintaining nitrogen balance and the effective reutilization of the other amino acids.[199,200] Therefore, the requirement for methionine increases significantly during times of high protein turnover, as seen in burn and trauma patients.[201,202] The dietary requirement for methionine, originally based on early nitrogen balance studies,[203,204] is usually reported as a component of the requirement for total sulfur amino acids (SAA) that includes cysteine/cystine. Estimates of adult SAA daily requirements range between 13 and 16 mg/kg (17 to 27 mg/g protein).[205]

Typical Use

- Exercisers/athletes seeking continuous physical and performance progress.
- Especially important recovery aid for older exercisers/athletes.
- Any dieter including users of intermittent fasting and ketogenic diets. Due to its low-calorie content and high anabolic potential, it may be used as the sole pre and post activity supplement for anyone requiring low

body fat, following prolonged restricted calorie diets, and/or dieting because of weight restrictions (e.g., weight classes).
- Ideal for recovery on days of multiple training sessions or tournament play before, during or after each event (i.e., trickle in with normal fluid/electrolyte intake).
- Can be used with NO7Rage™ and in the dotFIT™ "Stacking Programs," providing enhanced progressive exercise-induced results.
- All non-exercisers over 30 years of age.

Precautions

Presently, insufficient data exists to use the risk assessment model for determining an upper limit (UL) for any of the amino acids. Furthermore, chronic excessive use of individual amino acids is highly unlikely in athletes (no perceived value at levels that may lead to danger) and potentially uncomfortable (e.g., stomach distress). Consequently, collecting data on amino acid toxicity is difficult and possibly unnecessary. Reported adverse events from acute and chronic high-level intake of amino acids are extremely rare.[206] Amino acid supplementation safety appears to have survived the "test of time" as it relates to use by athletes. Despite the lack of adverse events reported by athletes who use amino acid products and the lack of UL values for amino acids, the safety of chronic high intakes of amino acids is unknown. However, the risk/benefit ratio appears to be extremely low. And the amounts present in recommended doses of AminoFormula do not approach any level of amino acid intake that may lead to adverse events. Phenylketonuria (PKU) is a rare disease (generally diagnosed at birth) caused by an inborn error in the ability to metabolize phenylalanine (lacking the enzyme phenylalanine hydroxylase).[207] In affected people, if the diet is not controlled by severe restriction of phenylalanine intake, PKU can lead to serious irreversible neurological disorders, such as mental retardation. Because homocysteinemia is linked with cardiovascular disease, long-term use of high intakes of individual methionine supplements may be of concern.[208]

Contraindications

This product, as with any protein/AA or creatine-containing supplement, is contraindicated for users with kidney or liver disease. This product is contraindicated for phenylketonurics because it contains phenylalanine. This product is also contraindicated for pregnant or lactating females because for ethical reasons it is not studied in these groups and because protein can be adequately supplied by the diet for fetal growth or lactation needs. BCAAs are contraindicated in people with the rare genetic disorder, Maple Syrup Disease, as they cannot properly process the AAs.[209]

Adverse Reactions

BCAAs have been used in studies in doses of at least 12 g/day with no side effects, making the dose in AminoFormula safe for healthy users.[210] Large doses of BCAAs ingested rapidly may cause stomach distress in sensitive users.[211]

Lysine is often used for herpes simplex at an oral dose of 1 to 3 g/day. It has been used in doses from 400 mg to 6 g/day without adverse events. Above 8 g/day, however, can cause profuse watery diarrhea in those with lysinuric protein intolerance.[212,213] These studies suggest the lysine in AminoFormula should be well tolerated in healthy users.[168]

Methionine: The average American consumes at least 2 grams (2,000 mg) of methionine each day.[214] 2.5 grams every four hours for 16 hours has been used for acetaminophen poisoning and for liver disorders.[215] Methionine is frequently used in doses of 100 mg/kg to test individuals with various diseases and levels of

homocysteine in their blood.[214,216] The 100 mg/kg dose is generally considered a safe dose during short-term use for medical testing with mild side effects reported.[217] 100 mg/kg is a much larger dose than users will receive in AminoFormula. Coincidentally, both methionine deficiency and an excessive acute intake (>100 mg/kg) are associated with liver diseases and other adverse events.[218,219] These studies suggest the methionine in AminoFormula should be well tolerated in healthy users.

Histidine supplementation in doses of 4 g/day has shown no side effects,[220] whereas doses of 24 to 64 g have caused anorexia and increased urinary zinc excretion.[221] These studies suggest the histidine in AminoFormula should be well tolerated in healthy users.

Threonine has been used for spinal spasticity and amyotrophic lateral sclerosis (Lou Gehrig's disease) in doses from 2 to 7.5 g/day.[219,222] Threonine in doses up to 4 g/day is associated with mild adverse events ranging from nothing to slight GI discomfort. One study of spasticity at 7.5 g/day showed no adverse events.[222] These studies suggest the threonine in AminoFormula should be well tolerated in healthy users.

Phenylalanine has been used for a depigmentation disorder called vitiligo in doses of up to 100 mg/kg with minimal to no side effects.[223] A typical 154-pound athlete would be consuming 7 g/day at that dose, making the amount in AminoFormula safe for users without PKU.

Overall, essential amino acid supplementation in combined doses from 12 to 60 g/day and as described here is well tolerated and exhibits a strong safety profile in individuals without PKU, kidney or liver disease.
41,44,47,48,49,52,53,54,75,76,77,86,87,90,91,92,93,94,95,96,106,107,108,110,111,150,151,152,153,154,155,156,157,158,159,160,162,224

Upper Limit/Toxicity

- Amino acid blends and protein supplements have been studied for use in numerous disease states and to improve sports performance for decades with a large margin of safety between the typical doses and those needed to elicit toxic effects in healthy users[21,22,54,55,57,162,206,208,224,225,226,227] including children.[228]
- A proposed upper limit for leucine (only based on plasma and urinary variables, not an adverse reaction) is ~227 mg/lb (150 lb person would need to consume ~34 g of leucine to approach this level) [229] We should be mindful that even at this proposed level there are no known side effects. It was simply not making a positive contribution in protein metabolism/synthesis in the subjects under the conditions of the study.
- In addition, the amino acids in AminoFormula all appear on the Generally Regarded as Safe (GRAS) list and are in forms which may be safely used when added to foods.[230]

Table 6: Toxicity in animals*

AA	NOAEL/LOAEL†	LD_{50} Mouse	LD_{50} Rat
Histidine	>4.5 g/d	15,000 mg/kg orally	15,000 mg/kg orally
Isoleucine	14.4 g BCAA/d		6822 mg/kg*
Leucine	>6 g/d		5379 mg/kg*
Lysine	3 – 40 g/d		10,000 mg/kg^
Methionine	5 g/d	9500 mg/kg*	36,000 mg/kg orally
Phenylalanine	>4 g/d	1322 mg/kg*	5287 mg/kg*
Threonine	>6 g/d		3098 mg/kg*
Valine	14.4 g BCAA/d		5390 mg/kg*

* Intraperitonea **Data from TOXNET; ChemIDplus Lite. ^ Data from MSDS sheets.
†Derived from Garlick et al.[208] Doses given are levels used in studies that showed mild to no adverse events and are clearly below toxic levels.

Summary

Purpose

Maximize MPS/recovery and control body composition while pursuing any sport and fitness goal

To supply proportionate to the demand, the primary signaling molecules, leucine, isoleucine, valine, histidine, phenylalanine, threonine, lysine and methionine that are directly responsible for triggering muscle protein synthesis (MPS) to deliver:

- An additive MPS effect to the daily ingestion of intact protein to optimize the user's exercise induced MPS/recovery potential to maximize and prolong training gains.
    - Fully recover and avoid plateaus by delivering a highly anabolic formula in extreme low calories in support of controlling body composition while pursuing muscle size and performance goals.
- For the non-exercising adult population, a low calorie MPS supplement to slow age-related loss of muscle and related aspects (e.g., balance, bone, strength, injury, etc.), that starts in the third or fourth decade of life.
- A low nitrogen and sulfur producing supplement in a flexible low calorie starting formula (add food/supplement components as desired) to support protein needs for anyone, which may be especially important to the rapidly expanding aging population hampered by age-related muscle loss combined with or without loss of appetite or diminishing organ functions.
- A pre and post activity supplement for exercisers during aggressive weight/fat loss including integration within a weight/bodyfat reduction meal replacement program.

Unique Features

- The formula uses a leucine enriched EAA blend that has been shown to significantly increase muscle protein synthesis in clinical trials with both adult athletes and non-athletes of all ages.
- The proprietary EAA composition supplies the anabolic agents of protein, in proportion to the demand, that are directly responsible for triggering muscle protein synthesis (MPS) in an isolated, low calorie and powdered form allowing timely, rapid, and relatively unobstructed transport to skeletal muscles to help maximize MPS.
- Extremely high anabolic formula delivered in a low calorie, relatively low nitrogen and very palatable drink/solution.
- Used with any program, this unique formula provides an incremental performance/muscle and recovery effect, thus can be additive to all other protocols including pre/post protein and carbohydrate feedings.
- NSF Certified for Sport (NSFCS).

Supplement Facts Panel

Strategic Essential Amino Acids, including BCAA's
Leucine (4,000 mg)
Isoleucine (900 mg)
Valine (1,100 mg)
Lysine (1670 mg)
Phenylalanine (1,670.5 mg)
Threonine (1300 mg)
Histidine (900 mg)
Methionine (360 mg)

References

[1] Sartorelli V., Fulco M. Molecular and cellular determinants of skeletal muscle hypertrophy. Sci STKE 2004, rell.

[2] Lopez MJ, Mohiuddin SS. Biochemistry, Essential Amino Acids. [Updated 2020 Apr 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557845/

[3] Nix, Staci. Williams' Basic Nutrition and Diet Therapy, 15th Edition. 2017 Elsevier Inc. Part 1, Chapters 4 Proteins, pgs. 41-55. ISBN: 978-0-323-37731-7

[4] Tan KT, Ang SJ, Tsai SY. Sarcopenia: Tilting the Balance of Protein Homeostasis. Proteomics. 2020 Mar;20(5-6):e1800411. doi: 10.1002/pmic.201800411. Epub 2019 Dec 4. PMID: 31722440.

[5] Morley JE, Baumgartner RN, Roubenoff R, Mayer J, Nair KS. Sarcopenia. J Lab Clin Med 2001; 137: 231–43

[6] Evans, W.J. Skeletal muscle loss: Cachexia, sarcopenia, and inactivity. *Am. J. Clin. Nutr.* 2010, *91*, 1123S–1127S

[7] Colon, Gabriela et al. "The enigmatic role of growth hormone in age-related diseases, cognition, and longevity". *GeroScience* vol. 41,6 (2019): 759-774. doi:10.1007/s11357-019-00096-w

[8] Nielsen J, Jensen RB, Afdeling AJ (2015) Growth hormone deficiency in children. Ugeskr Laeger 177(26):1260–1263

[9] Devesa J, Almenglo C, Devesa P (2016a) Multiple effects of growth hormone in the body: is it really the hormone for growth? Clin Med Insights Endocrinol Diabetes 9:47–71

[10] Phillips SM, Tipton KD, Aarsland A, et al. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am J Physiol. 1997;273:E99–107

[11] Moore, D.R.; Robinson, M.J.; Fry, J.L.; Tang, J.E.; Glover, E.I.; Wilkinson, S.B.; Prior, T.; Tarnopolsky, M.A.; Phillips, S.M. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am. J. Clin. Nutr.* 2009, *89*, 161–168

[12] Yang, Y.; Breen, L.; Burd, N.A.; Hector, A.J.; Churchward-Venne, T.A.; Josse, A.R.; Tarnopolsky, M.A.; Phillips, S.M. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *Br. J. Nutr.* 2012, doi:10.1017/S0007114511007422

[13] Larsson, Lars et al. "Sarcopenia: Aging-Related Loss of Muscle Mass and Function." *Physiological reviews* vol. 99,1 (2019): 427-511. doi:10.1152/physrev.00061.2017

[14] Joanisse, S., Lim, C., McKendry, J., Mcleod, J.C., Stokes, T., & Phillips, S.M. (2020). Recent advances in understanding resistance exercise training-induced skeletal muscle hypertrophy in humans. F1000 Research, 9, F1000 Faculty Rev-141. PubMed ID: 32148775 doi:10.12688/f1000research.21588.1

[15] Kim IY, Deutz NEP, Wolfe RR. Update on maximal anabolic response to dietary protein [review]. Clin Nutr. 2018;37(2):411–8.

[16] Mette Hansen, Jens Bangsbo, Jørgen Jensen, Bo Martin Bibby, and Klavs Madsen. Effect of Whey Protein Hydrolysate on Performance and Recovery of Top-Class Orienteering Runners. DOI: http://dx.doi.org/10.1123/ijsnem.2014-0083

[17] Stuart M. Phillips. A Brief Review of Critical Processes in Exercise-Induced Muscular Hypertrophy. Sports Med (2014) 44 (Suppl 1):S71–S77. DOI 10.1007/s40279-014-0152-3

[18] Stefan M. Pasiakos. Exercise and Amino Acid Anabolic Cell Signaling and the Regulation of Skeletal Muscle Mass. *Nutrients* 2012, *4*, 740-758; doi:10.3390/nu4070740

[19] Optimizing Protein Intake in Adults: Interpretation and Application of the Recommended Dietary Allowance Compared with the Acceptable Macronutrient Distribution Range. *Adv Nutr*. 2017;8(2):266-275. Published 2017 Mar 15. doi:10.3945/an.116.013821

[20] Wirth J, Hillesheim E, Brennan L. The Role of Protein Intake and its Timing on Body Composition and Muscle Function in Healthy Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. J Nutr. 2020 Jun 1;150(6):1443-1460. doi: 10.1093/jn/nxaa049. PMID: 32232404

[21] Kerksick, Chad M et al. "ISSN exercise & sports nutrition review update: research & recommendations." *Journal of the International Society of Sports Nutrition* vol. 15,1 38. 1 Aug. 2018, doi:10.1186/s12970-018-0242-y

[22] Antonio J. High-protein diets in trained individuals. Res Sports Med. 2019 Apr-Jun;27(2):195-203. doi: 10.1080/15438627.2018.1523167. Epub 2018 Sep 22. PMID: 30244609.

[23] Ahtiainen, J.P., Pakarinen, A., Alen, M., Kraemer, W.J., & Hakkinen, K. (2003). Muscle hypertrophy, hormonal adaptations and strength development during strength training in strength-trained and untrained men. European Journal of Applied Physiology, 89(6), 555–563. PubMed ID: 12734759 doi:10.1007/s00421-003-0833-3

[24] Flann, K.L., LaStayo, P.C., McClain, D.A., Hazel, M., & Lindstedt, S.L. (2011). Muscle damage and muscle remodeling: No pain, no gain? The Journal of Experimental Biology, 214(Pt. 4), 674–679. PubMed ID: 21270317 doi:10.1242/jeb.050112
[25] Ribeiro, A.S., Tomeleri, C.M., Souza, M.F., Pina, F.L., Schoenfeld, B.J., Nascimento, M.A., : : : Cyrino, E.S. (2015). Effect of resistance training on C-reactive protein, blood glucose and lipid profile in older women with differing levels of RT experience. Age, 37(6), 109. PubMed ID: 26499819 doi:10.1007/s11357-015-9849-y
[26] Desbrow et al. Nutrition for Special Populations: Young, Female, and Masters Athletes. International Journal of Sport Nutrition and Exercise Metabolism, 2019, 29, 220-227. https://doi.org/10.1123/ijsnem.2018-0269
[27] Philp A, Hamilton DL, Baar K. Signals mediating skeletal muscle remodeling by resistance exercise: PI3-kinase independent activation of mTORC1. J Appl Physiol. 2011;110:561–8
[28] Dickinson JM, Gundermann DM, Walker DK, Reidy PT, Borack MS, Drummond MJ, Arora M, Volpi E, Rasmussen BB. Leucine-enriched amino acid ingestion after resistance exercise prolongs myofibrillar protein synthesis and amino acid transporter expression in older men. J Nutr. 2014 Nov;144(11):1694-702. doi: 10.3945/jn.114.198671. Epub 2014 Sep 3
[29] Vinod Kumar, Philip Atherton, Kenneth Smith, Michael J. Rennie. Human muscle protein synthesis and breakdown during and after exercise. Journal of Applied Physiology Published 1 June 2009 Vol. 106 no. 6, 2026-2039 DOI:10.1152/japplphysiol.91481.2008
[30] Cheng S, Degens H, Evans M, Cheng SM, Selänne H, Rittweger J, Heinonen A, Suominen H, Strandberg T, Alen M, Korhonen MT Gerontology What Makes a 97-Year-Old Man Cycle 5,000 km a Year? *2016; 62(5):508-12.*
[31] Korhonen MT, Cristea A, Alén M, Häkkinen K, Sipilä S, Mero A, Viitasalo JT, Larsson L, Suominen H. Aging, muscle fiber type, and contractile function in sprint-trained athletes. *J Appl Physiol (1985). 2006 Sep; 101(3):906-17.*
[32] Wall BT, Gorissen SH, Pennings B, Koopman R, Groen BB, Verdijk LB, van Loon LJ. Aging Is Accompanied by a Blunted Muscle Protein Synthetic Response to Protein Ingestion. *PLoS One. 2015; 10(11):e0140903.*
[33] Aquilani R, D'Antona G, Baiardi P, Gambino A, Iadarola P, Viglio S, Pasini E, Verri M, Barbieri A, Boschi F. Essential amino acids and exercise tolerance in elderly muscle depleted subjects with chronic diseases: a rehabilitation without rehabilitation? Biomed Res Int. 2014;2014:341603. doi: 10.1155/2014/341603. Epub 2014 Jun 9
[34] Liao et al. Review. Prospective Views for Whey Protein and/or Resistance Training Against Age-related Sarcopenia. Aging and Disease. Volume 10, Number 1; 158-174. February 2019
[35]Stecker et al. Timing of ergogenic aids and micronutrients on muscle and exercise performance. Journal of the International Society of Sports Nutrition (2019) 16:37 https://doi.org/10.1186/s12970-019-0304-9
[36] Arent SM, Cintineo HP, McFadden BA, Chandler AJ, Arent MA. Nutrient Timing: A Garage Door of Opportunity? Nutrients. 2020 Jun 30;12(7):1948. doi: 10.3390/nu12071948. PMID: 32629950; PMCID: PMC7400240.
[37] Morton, Robert W et al. "A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults." *British journal of sports medicine* vol. 52,6 (2018): 376-384. doi:10.1136/bjsports-2017-097608
[38] Fujita S, Dreyer HC, Drummond MJ, Glynn EL, Cadenas JG, Yoshizawa F, Volpi E, Rasmussen BB. Nutrient signaling in the regulation of human muscle protein synthesis. J Physiol 582: 813–823, 2007. [PMCID: PMC2075348] [PubMed: 17478528]
[39] Atherton, P.J.; Etheridge, T.; Watt, P.W.; Wilkinson, D.; Selby, A.; Rankin, D.; Smith, K.; Rennie, M.J. Muscle full effect after oral protein: Time-dependent concordance and discordance between human muscle protein synthesis and mTORC1 signaling. *Am. J. Clin. Nutr.* 2010, *92*, 1080–1088
[40] Paddon-Jones D, Sheffield-Moore M, Katsanos CS, Zhang XJ, Wolfe RR. Differential stimulation of muscle protein synthesis in elderly humans following isocaloric ingestion of amino acids or whey protein. Exp Gerontol. 2006;41(2):215–9.
[41] Park, Sanghee et al. "Anabolic response to essential amino acid plus whey protein composition is greater than whey protein alone in young healthy adults." *Journal of the International Society of Sports Nutrition* vol. 17,1 9. 10 Feb. 2020, doi:10.1186/s12970-020-0340-5
[42] Drummond, M.J.; Rasmussen, B.B. Leucine-enriched nutrients and the regulation of mammalian target of rapamycin signaling and human skeletal muscle protein synthesis. *Curr. Opin. Clin. Nutr. Metab. Care* 2008, *11*, 222–226
[43] Bird, S.P.; Tarpenning, K.M.; Marino, F.E. Independent and combined effects of liquid carbohydrate/essential amino acid ingestion on hormonal and muscular adaptations following resistance training in untrained men. *Eur. J. Appl. Physiol*. 2006, *97*, 225–238
[44] Bird, S.P.; Tarpenning, K.M.; Marino, F.E. Liquid carbohydrate/essential amino acid ingestion during a short-term bout of resistance exercise suppresses myofibrillar protein degradation. *Metabolism. Clin. Exp*. 2006, *55*, 570–577

[45] Hodson N, West DWD, Philp A, *et al.*: Molecular regulation of human skeletal muscle protein synthesis in response to exercise and nutrients: a compass for overcoming age-related anabolic resistance. *Am J Physiol Cell Physiol.* 2019; 317(6): C1061–C1078.
[46] You JS, McNally RM, Jacobs BL, *et al.*: The role of raptor in the mechanical load-induced regulation of mTOR signaling, protein synthesis, and skeletal muscle hypertrophy. *FASEB J.* 2019; 33(3): 4021–34.
[47] Bohé J, Low A, Wolfe RR, Rennie MJ. Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose-response study. J Physiol. 2003 Oct 1;552(Pt 1):315-24. Epub 2003 Aug 8.
[48] Daniel WD West, Nicholas A Burd, Vernon G Coffey, Steven K Baker, Louise M Burke, John A Hawley, Daniel R Moore, Trent Stellingwerff, and Stuart M Phillips. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. Am J Clin Nutr 2011;94:795–803. Printed in USA. _ 2011 American Society for Nutrition
[49] Churchward-Venne, T.A.; Burd, N.A.; Phillips, S.M. Nutritional regulation of muscle protein synthesis with resistance exercise: Strategies to enhance anabolism. Nutr. Metab. 2012, 9, 40.
[50] Gowans G. J., , Hardie D. G. (2014) AMPK: a cellular energy sensor primarily regulated by AMP. Biochem. Soc. Trans. 42, 71–75 10.1042/BST20130244 [PMCID: PMC5703408] [
[51] Kjøbsted, Rasmus et al. "AMPK in skeletal muscle function and metabolism." *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* vol. 32,4 (2018): 1741-1777. doi:10.1096/fj.201700442R
[52] Glynn EL, Fry CS, Drummond MJ, Dreyer HC, Dhanani S, Volpi E, Rasmussen BB. Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise. Am J Physiol Regul Integr Comp Physiol. 2010 Aug;299(2):R533-40. Epub 2010 Jun 2.
[53] Gwin JA, Church DD, Hatch-McChesney A, Howard EE, Carrigan CT, Murphy NE, Wilson MA, Margolis LM, Carbone JW, Wolfe RR, Ferrando AA, Pasiakos SM. Effects of high versus standard essential amino acid intakes on whole-body protein turnover and mixed muscle protein synthesis during energy deficit: A randomized, crossover study. Clin Nutr. 2021 Mar;40(3):767-777. doi: 10.1016/j.clnu.2020.07.019. Epub 2020 Jul 22. PMID: 32768315.
[54] Spruce, Neal F., and Alan C. Titchenal. "Other Individual Amino Acids." *Sports Nutrition Fats and Proteins.* Judy A. Hillsdale. Boca Raton, FL: CRC Press, 2007. 279-333. Print
[55] Apex Fitness website/data base 1994-2008 and dotFIT website/data bases 2009-present
[56] Plotkin DL, Delcastillo K, Van Every DW, Tipton KD, Aragon AA, Schoenfeld BJ. Isolated Leucine and Branched-Chain Amino Acid Supplementation for Enhancing Muscular Strength and Hypertrophy: A Narrative Review. Int J Sport Nutr Exerc Metab. 2021 Mar 18:1-10. doi: 10.1123/ijsnem.2020-0356. Epub ahead of print. PMID: 33741748.
[57] Jäger R, Kerksick CM, Campbell BI, et al. International Society of Sports Nutrition Position Stand: protein and exercise. *J Int Soc Sports Nutr.* 2017;14:20. Published 2017 Jun 20. doi:10.1186/s12970-017-0177-8
[58] Kamei, Yasutomi et al. "Regulation of Skeletal Muscle Function by Amino Acids." *Nutrients* vol. 12,1 261. 19 Jan. 2020, doi:10.3390/nu12010261
[59] Drummond, M. et al. "Regulation of Protein Metabolism in Exercise and Recovery Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC 1 signaling." (2009).
[60] Byfield, M.P.; Murray, J.T.; Backer, J.M. hVps34 is a nutrient-regulated lipid kinase required for activation of p70 S6 kinase. *J. Biol. Chem.* 2005, *280*, 33076–33082
[61] Nobukuni, T.; Joaquin, M.; Roccio, M.; Dann, S.G.; Kim, S.Y.; Gulati, P.; Byfield, M.P.; Backer, J.M.; Natt, F.; Bos, J.L.; *et al.* Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase. *Proc. Natl. Acad. Sci. USA* 2005, *102*, 14238–14243
[62] MacKenzie, M.G.; Hamilton, D.L.; Murray, J.T.; Taylor, P.M.; Baar, K. mVps34 is activated following high-resistance contractions. *J. Physiol.* 2009, *587*, 253–260.
[63] Takegaki, Junya et al. "The Effect of Leucine-Enriched Essential Amino Acid Supplementation on Anabolic and Catabolic Signaling in Human Skeletal Muscle after Acute Resistance Exercise: A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Comparison Trial." *Nutrients* vol. 12,8 2421. 12 Aug. 2020, doi:10.3390/nu12082421
[64] Drummond, M.J.; Fry, C.S.; Glynn, E.L.; Timmerman, K.L.; Dickinson, J.M.; Walker, D.K.; Gundermann, D.M.; Volpi, E.; Rasmussen, B.B. Skeletal muscle amino acid transporter expression is increased in young and older adults following resistance exercise. *J. Appl. Physiol.* 2011, *111*, 135–142

[65] Drummond, M.J.; Glynn, E.L.; Fry, C.S.; Timmerman, K.L.; Volpi, E.; Rasmussen, B.B. An increase in essential amino acid availability upregulates amino acid transporter expression in human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 2010, *298*, E1011–E1018
[66] Churchward-Venne, T.A.; Burd, N.A.; Mitchell, C.J.; West, D.W.; Philp, A.; Marcotte, G.R.; Baker, S.K.; Baar, K.; Phillips, S.M. Supplementation of a suboptimal protein dose with leucine or essential amino acids: Effects on myofibrillar protein synthesis at rest and following resistance exercise in men. *J. Physiol.* 2012, *590*, 2751–2765
[67] Ogasawara R, Kobayashi K, Tsutaki A, Lee K, Abe T, Fujita S, Nakazato K, Ishii N. mTOR signaling response to resistance exercise is altered by chronic resistance training and detraining in skeletal muscle. J Appl Physiol (1985) 114: 934–940, 2013. doi:10.1152/japplphysiol.01161.2012.
[68] Terzis G, Georgiadis G, Stratakos G, Vogiatzis I, Kavouras S, Manta P, Mascher H, Blomstrand E. Resistance exercise-induced increase in muscle mass correlates with p70S6 kinase phosphorylation in human subjects. Eur J Appl Physiol 102: 145–152, 2008. doi:10.1007/s00421-007-0564-y.
[69] Townsend, Jeremy R et al. "The Effect of ProHydrolase® on the Amino Acid and Intramuscular Anabolic Signaling Response to Resistance Exercise in Trained Males." *Sports (Basel, Switzerland)* vol. 8,2 13. 22 Jan. 2020, doi:10.3390/sports8020013
[70] Kendall J. Condon et al. Nutrient regulation of mTORC1 at a glance 2019. Published by The Company of Biologists Ltd. Journal of Cell Science (2019) 132, jcs222570. doi:10.1242/jcs.222570
[71] Volpi E, Kobayashi H, Sheffield-Moore M, et al. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr. 2003;78:250–8.
[72] Tipton KD, Ferrando AA, Phillips SM, Doyle D Jr, Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol. 1999 Apr;276(4 Pt 1):E628-34.
[73] Tipton, K.D.; Gurkin, B.E.; Matin, S.; Wolfe, R.R. Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. *J. Nutr. Biochem.* 1999, *10*, 89–95
[74] Deutz, N.E.P.;Wolfe, R.R. Is there a maximal anabolic response to protein intake with a meal? Clin. Nutr. 2012, 32, 309–313
[75] Rennie MJ, Bohé J, Smith K, Wackerhage H, Greenhaff P. Branched-chain amino acids as fuels and anabolic signals in human muscle. J Nutr. 2006 Jan;136(1 Suppl):264S-8S. Review.
[76] Yoshizawa F. Regulation of protein synthesis by branched-chain amino acids in vivo. Biochem Biophys Res Commun. 2004 Jan 9;313(2):417-22. Review.
[77] Tipton KD, Elliott TA, Cree MG, Aarsland AA, Sanford AP, Wolfe RR. Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. Am J Physiol Endocrinol Metab. 2007 Jan;292(1):E71-6. Epub 2006 Aug 8.
[78] Biolo, G.; Fleming, R.Y.; Maggi, S.P.;Wolfe, R.R. Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. Am. J. Physiol. Metab. 1995, 268, E75–E84
[79] Tipton KD, Ferrando AA, Phillips SM, Doyle DJ, Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol 1999;276:E628-34
[80] Volpi, E.; Ferrando, A.A.; Yeckel, C.W.; Tipton, K.D.;Wolfe, R.R. Exogenous amino acids stimulate net muscle protein synthesis in the elderly. J. Clin. Investig. 1998, 101, 2000–2007
[81] Guimarães-Ferreira L, Cholewa JM, Naimo MA, Zhi XI, Magagnin D, de Sá RB, Streck EL, Teixeira Tda S, Zanchi NE. Synergistic effects of resistance training and protein intake: practical aspects. Nutrition. 2014 Oct;30(10):1097-103. doi: 10.1016/j.nut.2013.12.017. Epub 2014 Jan 10
[82] Robinson MM , Soop M, Sohn TS, Morse DM, Schimke JM, Klaus KA, Nair KS. High insulin combined with essential amino acids stimulates skeletal muscle mitochondrial protein synthesis while decreasing insulin sensitivity in healthy humans. J Clin Endocrinol Metab. 2014 Dec;99(12):E2574-83. doi: 10.1210/jc.2014-2736
[83] Carlin MB , Tanner RE , Agergaard J , Jalili T , McClain DA , Drummond MJ. Skeletal muscle Ras-related GTP binding B mRNA and protein expression is increased after essential amino acid ingestion in healthy humans. J Nutr. 2014 Sep;144(9):1409-14. doi: 10.3945/jn.114.196691. Epub 2014 Jul 23
[84] Proud, C.G. Amino acids and mTOR signalling in anabolic function. *Biochem. Soc. Trans.* 2007, *35*, 1187–1190
[85] Stipanuk, M.H. Leucine and protein synthesis: mTOR and beyond. *Nutr. Rev.* 2007, *65*, 122–129
[86] Greenhaff PL, Karagounis L, Peirce N, Simpson EJ, Hazell M, Layfield R, Wackerhage H, Smith K, Atherton P, Selby A, Rennie MJ. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin-ligases and protein turnover in human muscle. *Am J Physiol Endocrinol Metab* 295: E595–E604, 2008

[87] Beelen M, Koopman R, Gijsen AP, Vandereyt H, Kies AK, Kuipers H, Saris WH, van Loon LJ. Protein coingestion stimulates muscle protein synthesis during resistance-type exercise. *Am J Physiol Endocrinol Metab* 295: E70–E77,2008
[88] Matsui, Yasushi et al. "Effect of a leucine-enriched essential amino acids mixture on muscle recovery." *Journal of physical therapy science* vol. 31,1 (2019): 95-101. doi:10.1589/jpts.31.95
[89] MacLean DA, Graham TE, Saltin B. Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise. *Am J Physiol Endocrinol Metab* 267: E1010–E1022, 1994
[90] MacLean DA, Graham TE, Saltin B. Stimulation of muscle ammonia production during exercise following branched-chain amino acid supplementation in humans. *J Physiol* 493: 909–922, 1996
[91] Church DD, Hirsch KR, Park S, Kim IY, Gwin JA, Pasiakos SM, Wolfe RR, Ferrando AA. Essential Amino Acids and Protein Synthesis: Insights into Maximizing the Muscle and Whole-Body Response to Feeding. Nutrients. 2020 Dec 2;12(12):3717. doi: 10.3390/nu12123717. PMID: 33276485; PMCID: PMC7760188.
[92] Gwin, Jess A et al. "Muscle Protein Synthesis and Whole-Body Protein Turnover Responses to Ingesting Essential Amino Acids, Intact Protein, and Protein-Containing Mixed Meals with Considerations for Energy Deficit." *Nutrients* vol. 12,8 2457. 15 Aug. 2020, doi:10.3390/nu12082457
[93] Jonker R, Engelen MPKJ, Deutz NEP. Role of specific dietary amino acids in clinical conditions. Br J Nutr. 2012;108:S139–48.
[94] Church, D.D.; Gwin, J.A.; Wolfe, R.R.; Pasiakos, S.M.; Ferrando, A.A. Mitigation of Muscle Loss in Stressed Physiology: Military Relevance. Nutrients 2019, 11, 1703
[95] Pasiakos, S.M. Nutritional Requirements for Sustaining Health and Performance During Exposure to Extreme Environments. Annu. Rev. Nutr. 2020
[96] Phillips, S.M.; Paddon-Jones, D.; Layman, D.K. Optimizing Adult Protein Intake during Catabolic Health Conditions. Adv. Nutr. 2020, 11, S1058–S1069
[97] Smeets, J.S.J.; Horstman, A.M.H.; Vles, G.F.; Emans, P.J.; Goessens, J.P.B.; Gijsen, A.P.; Van Kranenburg, J.M.X.; Van Loon, L.J.C. Protein synthesis rates of muscle, tendon, ligament, cartilage, and bone tissue in vivo in humans. PLoS ONE 2019, 14, e0224745
[98] Nakshabendi, I.M.; McKee, R.; Downie, S.; Russell, R.I.; Rennie, M.J. Rates of small intestinal mucosal protein synthesis in human jejunum and ileum. Am. J. Physiol. 1999, 277, E1028–E1031.
[99] Tessari, P et al. "Kidney, splanchnic, and leg protein turnover in humans. Insight from leucine and phenylalanine kinetics." *The Journal of clinical investigation* vol. 98,6 (1996): 1481-92. doi:10.1172/JCI118937
[100] Wolfe, R.R.; Park, S.; Kim, I.Y.; Moughan, P.J.; Ferrando, A.A. Advances in stable isotope tracer methodology part 2: New thoughts about an "old" method-measurement of whole-body protein synthesis and breakdown in the fed state. J. Investig. Med. O_. Publ. Am. Fed. Clin. Res. 2020, 68, 11–15
[101] Sheffeld-Moore, M.;Wolfe, R.R.; Gore, D.C.;Wolf, S.E.; Ferrer, D.M.; Ferrando, A.A. Combined effects of hyper-aminoacidemia and oxandrolone on skeletal muscle protein synthesis. Am. J. Physiol. Endocrinol. Metab. 2000, 278, E273–E279
[102] Xu ZR , Tan ZJ , Zhang Q , Gui QF , Yang YMThe effectiveness of leucine on muscle protein synthesis, lean body mass and leg lean mass accretion in older people: a systematic review and meta-analysis. Br J Nutr. 2014 Sep 19:1-10. [Epub ahead of print]
[103] Alfonso J. Cruz-Jentoft, Francesco Landi, Stéphane M. Schneider, Clemente Zúñiga, et. al, Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS. Age and Ageing 2014; 43: 748–759 doi: 10.1093/ageing/afu115
[104] Kyle J. Hackney, and Kirk L. English. Protein and Essential Amino Acids to Protect Musculoskeletal Health during Spaceflight: Evidence of a Paradox?*Life* 2014, *4*, 295-317; doi:10.3390/life4030295
[105] Muyskens, Jonathan B et al. "Cellular and morphological changes with EAA supplementation before and after total knee arthroplasty." *Journal of applied physiology (Bethesda, Md. : 1985)* vol. 127,2 (2019): 531-545. doi:10.1152/japplphysiol.00869.2018
[106] Vieillevoye S, Poortmans JR, Duchateau J, Carpentier A. Effects of a combined essential amino acids/carbohydrate supplementation on muscle mass, architecture and maximal strength following heavy-load training. Eur J Appl Physiol. 2010 Oct;110(3):479-88. Epub 2010 Jun 3.

[107] Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, Prior T, Tarnopolsky MA, Phillips SM. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. Am J Clin Nutr. 2009 Jan;89(1):161-8. Epub 2008 Dec 3.
[108] Kreider RB, Campbell B. Protein for exercise and recovery. Phys Sportsmed. 2009 Jun;37(2):13-21. Review.
[109] Bukhari SS , Phillips BE , Wilkinson DJ , Limb MC , Rankin D , Mitchell WK , Kobayashi H , Greenhaff PL , Smith K , Atherton PJ. Intake of low-dose leucine-rich essential amino acids stimulates muscle anabolism equivalently to bolus whey protein in older women at rest and after exercise. Am J Physiol Endocrinol Metab. 2015 Jun 15;308(12):E1056-65. doi: 10.1152/ajpendo.00481.2014. Epub 2015 Mar 31
[110] Robert H Coker, Sharon Miller, Scott Schutzler, Nicolaas Deutz, and Robert R Wolfe. Whey protein and essential amino acids promote the reduction of adipose tissue and increased muscle protein synthesis during caloric restriction-induced weight loss in elderly, obese individuals. Nutr J. 2012; 11: 105. Published online 2012 December 11. doi: 10.1186/1475-2891-11-105PMCID: PMC3546025
[111] Gwin JA, Church DD, Hatch-McChesney A, Allen JT, Wilson MA, Varanoske AN, Carrigan CT, Murphy NE, Margolis LM, Carbone JW, Wolfe RR, Ferrando AA, Pasiakos SM. Essential amino acid-enriched whey enhances post-exercise whole-body protein balance during energy deficit more than iso-nitrogenous whey or a mixed-macronutrient meal: a randomized, crossover study. J Int Soc Sports Nutr. 2021 Jan 7;18(1):4. doi: 10.1186/s12970-020-00401-5. PMID: 33413462; PMCID: PMC7791816.
[112] Anthony J, Yoshizawa F, Anthony T, Vary T, Jefferson L, Kimball S. 2000. Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. J Nutr 130:2413–9.
[113] Lynch CJ, Halle B, Fujii H, Vary T, Wallin R, Damuni Z, Hutson S. Potential role of leucine metabolism in the leucine-signaling pathway involving mTOR. Am J Physiol Endocrinol Metab 2003;285:E854–63.
[114] Li F, Yin Y, Kong X, Wu G. Leucine nutrition in animals and humans: mTOR signaling and beyond. Amino Acids 2011;41:1185–93
[115] Kimball SR, Jefferson L. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. J Nutr 2006;136(Suppl):227S–31S
[116] Takegaki, Junya et al. "The Effect of Leucine-Enriched Essential Amino Acid Supplementation on Anabolic and Catabolic Signaling in Human Skeletal Muscle after Acute Resistance Exercise: A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Comparison Trial." *Nutrients* vol. 12,8 2421. 12 Aug. 2020, doi:10.3390/nu12082421
[117] Mobley, C Brooks et al. "Effects of Whey, Soy or Leucine Supplementation with 12 Weeks of Resistance Training on Strength, Body Composition, and Skeletal Muscle and Adipose Tissue Histological Attributes in College-Aged Males." *Nutrients* vol. 9,9 972. 4 Sep. 2017, doi:10.3390/nu9090972
[118] Churchward-Venne TA , Breen L, Di Donato DM, Hector AJ, Mitchell CJ, Moore DR, Stellingwerff T, Breuille D, Offord EA, Baker SK, Phillips SM Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: a double-blind, randomized trial. Am J Clin Nutr. 2014 Feb;99(2):276-86. doi: 10.3945/ajcn.113.068775. Epub 2013 Nov 27
[119] Tang JE[1], Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J Appl Physiol (1985). 2009 Sep;107(3):987-92. doi: 10.1152/japplphysiol.00076.2009. Epub 2009 Jul 9.
[120] Phillips, S.M. The impact of protein quality on the promotion of resistance exercise-induced changes in muscle mass. *Nutr Metab (Lond)* 13, 64 (2016). https://doi.org/10.1186/s12986-016-0124-8
[121] Devries MC , Phillips SM. Supplemental protein in support of muscle mass and health: advantage whey. J Food Sci. 2015 Mar;80 Suppl 1:A8-A15. doi: 10.1111/1750-3841.12802
[122] Tipton KD, Elliott TA, Ferrando AA, Aarsland AA, Wolfe RR. Stimulation of muscle anabolism by resistance exercise and ingestion of leucine plus protein. Appl Physiol Nutr Metab. 2009;34(2):151–61.
[123] Hiroyuki Kato, Hiromi Suzuki, Masako Mimura, et. Al, Leucine-enriched essential amino acids attenuate muscle soreness and improve muscle protein synthesis after eccentric contractions in rats. Amino Acids (2015) 47:1193–1201 DOI 10.1007/s00726-015-1946-9
[124] Dreyer HC et al (2008) Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. Am J Physiol Endocrinol Metab 294:E392–E400

[125] Stokes T, Hector AJ, Morton RW, McGlory C, Phillips SM. Recent Perspectives Regarding the Role of Dietary Protein for the Promotion of Muscle Hypertrophy with Resistance Exercise Training. Nutrients. 2018 Feb 7;10(2):180. doi: 10.3390/nu10020180. PMID: 29414855; PMCID: PMC5852756
[126]Rowlands DS , Nelson AR, Phillips SM, Faulkner JA, Clarke J, Burd NA, Moore D, Stellingwerff T. Protein-leucine fed dose effects on muscle protein synthesis after endurance exercise. Med Sci Sports Exerc. 2015 Mar;47(3):547-55. doi: 10.1249/MSS.0000000000000447
[127] B. Komar, L. Schwingshackl, Georg Hoffmann. Effects of leucine-rich protein supplements on anthropometric parameter and muscle strength in the elderly: A systematic review and meta-analysis. The journal of nutrition, health & aging. December 2014
[128] Christopher Brooks Mobley, Carlton D Fox, Brian S Ferguson, Rajesh H Amin, Vincent J Dalbo. L-leucine, beta-hydroxy-beta-methylbutyric acid (HMB) and creatine monohydrate prevent myostatin-induced Akirin-1/Mighty mRNA down-regulation and myotube atrophy. Mobley et al. Journal of the International Society of Sports Nutrition 2014, 11:38 http://www.jissn.com/content/11/1/38
[129] Luiking YC, et al., Protein type and caloric density of protein supplements modulate postprandial amino acid profile through changes in gastrointestinal behaviour: A randomized trial, Clinical Nutrition (2015), http://dx.doi.org/10.1016/j.clnu.2015.02.013
[130] Wolfe, R.R. Regulation of muscle protein by amino acids. *J. Nutr.* 2002, *132*, 3219S–3224S.
[131] Boirie Y. Fighting sarcopenia in older frail subjects: protein fuel for strength, exercise for mass. J Am Med Dir Assoc 2013;14(2):140e3.
[132] Hans C. Dreyer, Lisa A. Strycker, Hilary A. Senesac, et al, Essential amino acid supplementation in patients following total knee arthroplasty. *J Clin Invest.* 2013;123(11):4654–4666. doi:10.1172/JCI70160
[133] Waskiw-Ford M, Hannaian S, Duncan J, Kato H, Abou Sawan S, Locke M, Kumbhare D, Moore D. Leucine-Enriched Essential Amino Acids Improve Recovery from Post-Exercise Muscle Damage Independent of Increases in Integrated Myofibrillar Protein Synthesis in Young Men. Nutrients. 2020 Apr 11;12(4):1061. doi: 10.3390/nu12041061. PMID: 32290521; PMCID: PMC7231404.
[134] Negro et al. Essential Amino Acids (EAA) Mixture Supplementation: Effects of an Acute Administration Protocol on Myoelectric Manifestations of Fatigue in the Biceps Brachii After Resistance Exercise. Front. Physiol., 17 August 2018 https://doi.org/10.3389/fphys.2018.01140
[135] Hirsch KR, Greenwalt CE, Saylor HE, Gould LM, Harrison CH, Brewer GJ, Blue MNM, Ferrando AA, Huffman KM, Mayer-Davis EJ, Ryan ED, Smith-Ryan AE. High-intensity interval training and essential amino acid supplementation: Effects on muscle characteristics and whole-body protein turnover. Physiol Rep. 2021 Jan;9(1):e14655. doi: 10.14814/phy2.14655. PMID: 33369879; PMCID: PMC7769174.
[136] Coker, Robert H et al. "Essential amino acid-enriched meal replacement promotes superior net protein balance in older, overweight adults." *Clinical nutrition (Edinburgh, Scotland)* vol. 38,6 (2019): 2821-2826. doi:10.1016/j.clnu.2018.12.013
[137] Nygren J, Nair KS. Differential regulation of protein dynamics in splanchnic and skeletal muscle beds by insulin and amino acids in healthy human subjects. Diabetes 2003;52:1377e85.
[138] Krok-Schoen, J.L.; Archdeacon Price, A.; Luo, M.; Kelly, O.J.; Taylor, C.A. Low dietary protein intakes and associated dietary patterns and functional limitations in an aging population: A NHANES analysis. J. Nutr. Heal. Aging 2019, 23, 338–347
[139] Deutz NE , Bauer JM , Barazzoni R , Biolo G , Boirie Y , Bosy-Westphal, et al. Protein intake and exercise for optimal muscle function with aging: Recommendations from the ESPEN Expert Group. Clin Nutr. 2014 Apr 24. pii: S0261-5614(14)00111-3. doi: 10.1016/j.clnu.2014.04.007. [Epub ahead of print]
[140] Deer RR , Volpi E. Protein intake and muscle function in older adults. Curr Opin Clin Nutr Metab Care. 2015 May;18(3):248-53. doi: 10.1097/MCO.0000000000000162.
[141] Hruby, Adela et al. "Protein Intake and Functional Integrity in Aging: The Framingham Heart Study Offspring." *The journals of gerontology. Series A, Biological sciences and medical sciences* vol. 75,1 (2020): 123-130. doi:10.1093/gerona/gly201
[142] Otsuka R, Kato Y, Tange C, Nishita Y, Tomida M, Imai T, Ando F, Shimokata H, Arai H. Protein intake per day and at each daily meal and skeletal muscle mass declines among older community dwellers in Japan. Public Health Nutr. 2020 Apr;23(6):1090-1097. doi: 10.1017/S1368980019002921. Epub 2019 Oct 14. PMID: 31608843.

[143] Foscolou A, Magriplis E, Tyrovolas S, Chrysohoou C, Sidossis L, Matalas AL, Rallidis L, Panagiotakos D. The association of protein and carbohydrate intake with successful aging: a combined analysis of two epidemiological studies. Eur J Nutr. 2019 Mar;58(2):807-817. doi: 10.1007/s00394-018-1693-2. Epub 2018 Apr 23. PMID: 29687264
[144] Richter M, Baerlocher K, Bauer JM, Elmadfa I, Heseker H, Leschik-Bonnet E, Stangl G, Volkert D, Stehle P; on behalf of the German Nutrition Society (DGE). Revised Reference Values for the Intake of Protein. Ann Nutr Metab. 2019;74(3):242-250. doi: 10.1159/000499374. Epub 2019 Mar 22. PMID: 30904906; PMCID: PMC6492513
[145] Phillips SM, Chevalier S, Leidy HJ. Protein "requirements" beyond the RDA: implications for optimizing health. Appl Physiol Nutr Metab. 2016 May;41(5):565-72. doi: 10.1139/apnm-2015-0550. Epub 2016 Feb 9. PMID: 26960445.
[146] Franzke B, Neubauer O, Cameron-Smith D, Wagner KH. Dietary Protein, Muscle and Physical Function in the Very Old. Nutrients. 2018 Jul 20;10(7):935. doi: 10.3390/nu10070935. PMID: 30037048; PMCID: PMC6073115.
[147] Oliveira CLP, Dionne IJ, Prado CM. Are Canadian protein and physical activity guidelines optimal for sarcopenia prevention in older adults? Appl Physiol Nutr Metab. 2018 Dec;43(12):1215-1223. doi: 10.1139/apnm-2018-0141. Epub 2018 Jul 10. PMID: 29990445.
[148] Traylor DA, Gorissen SHM, Phillips SM. Perspective: Protein Requirements and Optimal Intakes in Aging: Are We Ready to Recommend More Than the Recommended Daily Allowance? Adv Nutr. 2018 May 1;9(3):171-182. doi: 10.1093/advances/nmy003. PMID: 29635313; PMCID: PMC5952928.
[149] Bauer J, Morley JE, Schols AMWJ, Ferrucci L, Cruz-Jentoft AJ, Dent E, Baracos VE, Crawford JA, Doehner W, Heymsfield SB, Jatoi A, Kalantar-Zadeh K, Lainscak M, Landi F, Laviano A, Mancuso M, Muscaritoli M, Prado CM, Strasser F, von Haehling S, Coats AJS, Anker SD. Sarcopenia: A Time for Action. An SCWD Position Paper. J Cachexia Sarcopenia Muscle. 2019 Oct;10(5):956-961. doi: 10.1002/jcsm.12483. Epub 2019 Sep 15. PMID: 31523937; PMCID: PMC6818450.
[150] Lees, Matthew J et al. “Novel Essential Amino Acid Supplements Following Resistance Exercise Induce Aminoacidemia and Enhance Anabolic Signaling Irrespective of Age: A Proof-of-Concept Trial.” *Nutrients* vol. 12,7 2067. 12 Jul. 2020, doi:10.3390/nu12072067
[151] Ispoglou, Theocharis et al. “Novel essential amino acid supplements enriched with L-leucine facilitate increased protein and energy intakes in older women: a randomised controlled trial.” *Nutrition journal* vol. 16,1 75. 28 Nov. 2017, doi:10.1186/s12937-017-0298-6
[152] Moro T, Brightwell CR, Deer RR, Graber TG, Galvan E, Fry CS, Volpi E, Rasmussen BB. Muscle Protein Anabolic Resistance to Essential Amino Acids Does Not Occur in Healthy Older Adults Before or After Resistance Exercise Training. J Nutr. 2018 Jun 1;148(6):900-909. doi: 10.1093/jn/nxy064. Erratum in: J Nutr. 2018 Oct 1;148(10):1699. PMID: 29796648; PMCID: PMC6251608.
[153] Ispoglou, T.; White, H.; Preston, T.; McElhone, S.; McKenna, J.; Hind, K. Double-blind, placebo-controlled pilot trial of L-Leucine-enriched amino-acid mixtures on body composition and physical performance in men and women aged 65–75 years. Eur. J. Clin. Nutr. 2016, 70, 182–188
[154] Negro M, Perna S, Spadaccini D, Castelli L, Calanni L, Barbero M, Cescon C, Rondanelli M, D'Antona G. Effects of 12 Weeks of Essential Amino Acids (EAA)-Based Multi-Ingredient Nutritional Supplementation on Muscle Mass, Muscle Strength, Muscle Power and Fatigue in Healthy Elderly Subjects: A Randomized Controlled Double-Blind Study. J Nutr Health Aging. 2019;23(5):414-424. doi: 10.1007/s12603-019-1163-4. PMID: 31021358.
[155] Bauer, J.M.; Verlaan, S.; Bautmans, I.; Brandt, K.; Donini, L.M.; Maggio, M.; McMurdo, M.E.; Mets, T.; Seal, C.; Wijers, S.L.; Ceda, G.P.; De Vito, G.; Donders, G.; et al. Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the PROVIDE study: a randomized, double-blind, placebo-controlled trial. J. Am. Med. Dir. Assoc. 2015, 16, 740-747, DOI: 10.1016/j.jamda.2015.05.021.
[156] Bell, K.E.; Snijders, T.; Zulyniak, M.; Kumbhare, D.; Parise, G.; Chabowski, A.; Phillips, S.M. A whey protein-based multi-ingredient nutritional supplement stimulates gains in lean body mass and strength in healthy older men: A randomized controlled trial. PLoS One. 2017, 12, e0181387, DOI: 10.1371/journal.pone.0181387.
[157] Yoshimura Y, Bise T, Shimazu S, Tanoue M, Tomioka Y, Araki M, Nishino T, Kuzuhara A, Takatsuki F. Effects of a leucine-enriched amino acid supplement on muscle mass, muscle strength, and physical function in post-stroke patients with sarcopenia: A randomized controlled trial. Nutrition. 2019 Feb;58:1-6. doi: 10.1016/j.nut.2018.05.028. Epub 2018 Jul 11. PMID: 30273819.
[158] Markofski MM, Jennings K, Timmerman KL, Dickinson JM, Fry CS, Borack MS, Reidy PT, Deer RR, Randolph A, Rasmussen BB, Volpi E. Effect of Aerobic Exercise Training and Essential Amino Acid Supplementation for 24 Weeks on Physical Function, Body Composition, and Muscle Metabolism in Healthy, Independent Older Adults: A Randomized Clinical Trial. J

Gerontol A Biol Sci Med Sci. 2019 Sep 15;74(10):1598-1604. doi: 10.1093/gerona/gly109. PMID: 29750251; PMCID: PMC6748753.
[159] Suzuki H, Yamashiro D, Ogawa S, Kobayashi M, Cho D, Iizuka A, Tsukamoto-Yasui M, Takada M, Isokawa M, Nagao K, Fujiwara Y. Intake of Seven Essential Amino Acids Improves Cognitive Function and Psychological and Social Function in Middle-Aged and Older Adults: A Double-Blind, Randomized, Placebo-Controlled Trial. Front Nutr. 2020 Nov 25;7:586166. doi: 10.3389/fnut.2020.586166. PMID: 33324669; PMCID: PMC7724102.
[160] Fernando W, Rainey-Smith SR, Gardener SL, Villemagne VL, Burnham SC, Macaulay SL, et al. Group: associations of dietary protein and fiber intake with brain and blood amyloid-beta. J Alzheimers Dis. (2018) 61:1589–98. doi: 10.3233/JAD-170742
[161] J-NHNS. The National Health and Nutrition Survey in Japan, 2018. Tokyo: Office of Nutrition; Ministry of Health, Labour andWelfare (2020).
[162] Borsheim E, Tipton KD, Wolf SE, Wolfe RR. Essential amino acids and muscle protein recovery from resistance exercise. Am J Physiol Endocrinol Metab 2002;283:E648
[163] Paddon-Jones D, Sheffield-Moore M, Aarsland A, Wolfe RR, Ferrando AA. Exogenous amino acids stimulate human muscle anabolism without interfering with the response to mixed meal ingestion. *Am J* Physiol Endocrinol Metab 2005;288:E761–E767
[164] Ohtani M, Maruyama K, Suzuki S, Sugita M, Kobayashi K. Changes in hematological parameters of athletes after receiving daily dose of a mixture of 12 amino acids for one month during the middle- and long-distance running training. Biosci Biotechnol Biochem 2001;65:348–355
[165] Ohtani M, Maruyama K, Sugita M, Kobayashi K. Amino acid supplementation affects hematological and biochemical parameters in elite rugby players. Biosci Biotechnol Biochem 2001;65:1970–1976
[166] Andersen LL, Tufekovic G, Zebis MK, Crameri RM, Verlaan G, Kjaer M, Suetta C, Magnusson P, Aagaard P. The effect of resistance training combined with timed ingestion of protein on muscle fiber size and muscle strength. Metabolism 2005;54:151–156.
[167] Tipton KD, Borsheim E, Wolf SE, Sanford AP, Wolfe RR. Acute response of net muscle protein balance reflects 24-h balance after exercise and amino acid ingestion. Am J Physiol Endocrinol Metab 2003;284:E76
[168] Spruce and Titchenal. Lysine Chapter 11. Pages 171-196. Nutritional ergogenic aids. Edited by Wolinsky and Driskell. 2004 CRC Press LLC
[169] Gropper, S.S., Smith, J.L. and Groff, J.L. Advanced Nutrition and Human Metabolism, 4th ed., Wadsworth, Belmont, 2005, pp. 205-206.
[170] van Loon LJ, Saris WH, Verhagen H, Wagenmakers AJ. Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. Am J Clin Nutr. 2000 Jul;72(1):96-105.
[171] van Loon LJ, Saris WH, Kruijshoop M, Wagenmakers AJ. Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. Am J Clin Nutr. 2000 Jul;72(1):106-11.
[172] Fernstrom JD, Fernstrom MH. Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *J Nutr.* 2007;137:1539S–1548S
[173] [No authors listed] Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). Institute of Medicine. Food and Nutrition Board (FNB). Office of Disease Prevention and Health Promotion of the U.S. Department of Health and Human Services. 2005. P. 27. www.nap.edu.
[174] Whitney EN, Rolfes SR, editors. Understanding Nutrition. 7th Ed. Minneapolis/St. Paul: West Publishing; 1996. 1694 p. pp 197-226.
[175] Flodin NW. The metabolic roles, pharmacology, and toxicology of lysine. J Am Coll Nutr. 1997 Feb;16(1):7-21.
[176] Hall SL, Greendale GA. The relation of dietary vitamin C intake to bone mineral density: results from the PEPI study. Calcif Tissue Int. 1998 Sep;63(3):183-9.
[177] Unal, Mustafa et al. "The Role of Matrix Composition in the Mechanical Behavior of Bone." *Current osteoporosis reports* vol. 16,3 (2018): 205-215. doi:10.1007/s11914-018-0433-0
[178] Daniel Tomé, Cécile Bos, Lysine Requirement through the Human Life Cycle, *The Journal of Nutrition*, Volume 137, Issue 6, June 2007, Pages 1642S–1645S, https://doi.org/10.1093/jn/137.6.1642S
[179] Linder, Maria C. Nutritional Biochemistry and Metabolism, 2nd Edition. New York: Elsevier, 1991. Pg 93.

[180] [No authors listed] Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation. World Health Organ Tech Rep Ser. 1985;724:1-206.
[181] Borgonha S, Regan MM, Oh SH, Condon M, Young VR. Threonine requirement of healthy adults, derived with a 24-h indicator amino acid balance technique. Am J Clin Nutr. 2002 Apr;75(4):698-704.
[182] Kurpad AV, Raj T, Regan MM, Vasudevan J, Caszo B, Nazareth D, Gnanou J, Young VR. Threonine requirements of healthy Indian men, measured by a 24-h indicator amino acid oxidation and balance technique. Am J Clin Nutr. 2002 Oct;76(4):789-97.
[183] Kriengsinyos W, Rafii M, Wykes LJ, Ball RO, Pencharz PB. Long-term effects of histidine depletion on whole-body protein metabolism in healthy adults. J Nutr. 2002 Nov;132(11):3340-8.
[184] Johnson P, Hammer JL. Histidine dipeptide levels in ageing and hypertensive rat skeletal and cardiac muscles. Comp Biochem Physiol B. 1992 Dec;103(4):981-4.
[185] Guiotto A, Calderan A, Ruzza P, Borin G. Carnosine and carnosine-related antioxidants: a review. Curr Med Chem. 2005;12(20):2293-315. Review.
[186] Begum G, Cunliffe A, Leveritt M. Physiological role of carnosine in contracting muscle. Int J Sport Nutr Exerc Metab. 2005 Oct;15(5):493-514. Review.
[187] Eric T. Trexler, Abbie E. Smith-Ryan, Jeffrey R. Stout, Jay R. Hoffman, Colin D. Wilborn, Craig Sale, Richard B. Kreider, et al. International society of sports nutrition position stand: Beta-Alanine. Trexler et al. Journal of the International Society of Sports Nutrition (2015) 12:30 DOI 10.1186/s12970-015-0090-y
[188] Kraemer WJ, Gordon SE, Lynch JM, Pop ME, Clark KL. Effects of multibuffer supplementation on acid-base balance and 2,3-diphosphoglycerate following repetitive anaerobic exercise. Int J Sport Nutr. 1995 Dec;5(4):300-14.
[189] Suzuki Y, Nakao T, Maemura H, Sato M, Kamahara K, Morimatsu F, Takamatsu K. Carnosine and anserine ingestion enhances contribution of nonbicarbonate buffering. Med Sci Sports Exerc. 2006 Feb;38(2):334-8.
[190] Suzuki Y, Ito O, Mukai N, Takahashi H, Takamatsu K. High level of skeletal muscle carnosine contributes to the latter half of exercise performance during 30-s maximal cycle ergometer sprinting. Jpn J Physiol. 2002 Apr; 52 (2):199-205.
[191] Moro, Joanna et al. "Histidine: A Systematic Review on Metabolism and Physiological Effects in Human and Different Animal Species." *Nutrients* vol. 12,5 1414. 14 May. 2020, doi:10.3390/nu12051414
[192] Fukagawa NK, Galbraith RA. Advancing age and other factors influencing the balance between amino acid requirements and toxicity. J Nutr. 2004 Jun;134(6 Suppl):1569S-1574S.
[193] Trimble KC, Molloy AM, Scott JM, Weir DG. The effect of ethanol on one-carbon metabolism: increased methionine catabolism and lipotrope methyl-group wastage. Hepatology. 1993 Oct;18(4):984-9.
[194] Duranton B, Freund JN, Galluser M, Schleiffer R, Gosse F, Bergmann C, Hasselmann M, Raul F. Promotion of intestinal carcinogenesis by dietary methionine. Carcinogenesis. 1999 Mar;20(3):493-7.
[195] Humm A, Fritsche E, Steinbacher S, Huber R. Crystal structure and mechanism of human L-arginine:glycine amidinotransferase: a mitochondrial enzyme involved in creatine biosynthesis. EMBO J. 1997 Jun 16;16(12):3373-85.
[196] Vaz FM, Wanders RJ. Carnitine biosynthesis in mammals. Biochem J. 2002 Feb 1;361(Pt 3):417-29. Review.
[197] Storch KJ, Wagner DA, Burke JF, Young VR. [1-13C; methyl-2H3]methionine kinetics in humans: methionine conservation and cystine sparing. Am J Physiol. 1990 May;258(5 Pt 1):E790-8.
[198] Storch KJ, Wagner DA, Burke JF, Young VR. Quantitative study in vivo of methionine cycle in humans using [methyl-2H3]- and [1-13C]methionine. Am J Physiol. 1988 Sep;255(3 Pt 1):E322-31.
[199] Kien CL, Young VR, Rohrbaugh DK, Burke JF. Increased rates of whole body protein synthesis and breakdown in children recovering from burns. Ann Surg. 1978 Apr;187(4):383-91.
[200] Yoshida, A. & Moritoki, K. Nitrogen sparing action of methionine and threonine in rats receiving a protein free diet. Nutrition Reports International. 1974: 9, 159-168.
[201] Millward DJ, Rivers JP. The need for indispensable amino acids: the concept of the anabolic drive. Diabetes Metab Rev. 1989 Mar;5(2):191-211. Review.
[202] Yokogoshi H, Yoshida A. Some factors affecting the nitrogen sparing action of methionine and threonine in rats fed a protein free diet. J Nutr. 1976 Jan;106(1):48-57.
[203] Rose WC, Wixom RL. The amino acid requirements of man. XII. The sparing effect of cysteine on the methionine requirements. *J Biol Chem.* 1955;216:763–74
[204] David H. Baker, Comparative Species Utilization and Toxicity of Sulfur Amino Acids, *The Journal of Nutrition*, Volume 136, Issue 6, June 2006, Pages 1670S–1675S, https://doi.org/10.1093/jn/136.6.1670S

[205] Young VR, Borgonha S. Nitrogen and amino acid requirements: : the Massachusetts Institute of Technology amino acid requirement pattern. J Nutr. 2000 Jul;130(7):1841S-9S. doi: 10.1093/jn/130.7.1841S. PMID: 10867061.
[206] "Safety Information." 2006. Food and Drug Administration. http://www.fda.gov/medwatch/.
[207] Garret RH, Grisham CM. Biochemistry, 3rd Edition. Belmont, CA: Thomson Brooks/Cole, 2005.
[208] Garlick PJ. The nature of human hazards associated with excessive intake of amino acids. J Nutr. 2004 Jun;134(6 Suppl):1633S-1639S; discussion 1664S-1666S, 1667S-1672S. Review.
[209] Simon E, Flaschker N, Schadewaldt P, Langenbeck U, Wendel U. Variant maple syrup urine disease (MSUD) – the entire spectrum. J Inherit Metab Dis. 2006;29:716-24
[210] Tang FC. Influence of branched-chain amino acid supplementation on urinary protein metabolite concentrations after swimming. J Am Coll Nutr. 2006 Jun;25(3):188-94.
[211] Gluud LL, Dam G, Les I, Córdoba J, Marchesini G, Borre M, Aagaard NK, Vilstrup H. Branched-chain amino acids for people with hepatic encephalopathy. Cochrane Database Syst Rev. 2015 Sep 17;(9):CD001939. doi: 10.1002/14651858.CD001939.pub3. Update in: Cochrane Database Syst Rev. 2017 May 18;5:CD001939. PMID: 26377410.
[212] Lukkarinen M, Nanto-Salonen K, Pulkki K, Aalto M, Simell O. Oral supplementation corrects plasma lysine concentrations in lysinuric protein intolerance. Metabolism. 2003 Jul;52(7):935-8.
[213] Corpas E, Blackman MR, Roberson R, Scholfield D, Harman SM. Oral arginine-lysine does not increase growth hormone or insulin-like growth factor-I in old men. J Gerontol. 1993 Jul;48(4):M128-33.
[214] Cottington EM, LaMantia C, Stabler SP, Allen RH, Tangerman A, Wagner C, Zeisel SH, Mudd SH. Adverse event associated with methionine loading test: a case report. Arterioscler Thromb Vasc Biol. 2002 Jun 1;22(6):1046-50.
[215] Smilkstein MJ, Knapp GL, Kulig KW, Rumack BH. Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the national multicenter study (1976 to 1985) N Engl J Med. 1988 Dec 15;319(24):1557-62.
[216] Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM, Palma-Reis RJ, Boers GH, Sheahan RG, Israelsson B, Uiterwaal CS, Meleady R, McMaster D, Verhoef P, Witteman J, Rubba P, Bellet H, Wautrecht JC, de Valk HW, Sales Luis AC, Parrot-Rouland FM, Tan KS, Higgins I, Garcon D, Andria G, et al. Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA. 1997 Jun 11;277(22):1775-81.
[217] Garlick P. Toxicity of methionine in humans. J Nutr. 2006 Jun;136(6 Suppl):1722S-1725S. Review.
[218] Fukagawa NK, Galbraith RA. Advancing age and other factors influencing the balance between amino acid requirements and toxicity. J Nutr. 2004 Jun;134(6 Suppl):1569S-1574S.
[219] Trimble KC, Molloy AM, Scott JM, Weir DG. The effect of ethanol on one-carbon metabolism: increased methionine catabolism and lipotrope methyl-group wastage. Hepatology. 1993 Oct;18(4):984-9.
[220] Gerber DA, Tanenbaum L, Ahrens M. Free serum histidine levels in patients with rheumatoid arthritis and control subjects following an oral load of free L-histidine. Metabolism. 1976 Jun;25(6):655-7.
[221] Geliebter AA, Hashim SA, Van Itallie TB. Oral L-histidine fails to reduce taste and smell acuity but induces anorexia and urinary zinc excretion. Am J Clin Nutr. 1981 Jan;34(1):119-20.
[222] Hauser SL, Doolittle TH, Lopez-Bresnahan M, Shahani B, Schoenfeld D, Shih VE, Growdon J, Lehrich JR. An antispasticity effect of threonine in multiple sclerosis. Arch Neurol. 1992 Sep;49(9):923-6.
[223] Camacho F, Mazuecos J. Oral and topical L-phenylalanine, clobetasol propionate, and UVA/sunlight--a new study for the treatment of vitiligo. J Drugs Dermatol. 2002 Sep;1(2):127-31.
[224] Tipton KD, Ferrando AA, Phillips SM, Doyle DJ, Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol 1999;276:E628-34
[225] Mansoor O, Breuille D, Bechereau F, Buffiere C, Pouyet C, Beaufrere B, Vuichoud J, Van't-Of M, Obled C. Effect of an enteral diet supplemented with a specific blend of amino acid on plasma and muscle protein synthesis in ICU patients. Clin Nutr. 2006 Sep 21.
[226] BrownROCompherC; American Society for Parenteral and Enteral Nutrition Board of Directors. ASPEN clinical guidelines: nutrition support in adult acute and chronic renal failure. JPEN J Parenter Enteral Nutr. 2010;34(4):366–377.
[227] Patel et al. Protein Requirements for Critically Ill Patients With Renal and Liver Failure. Nutrition in Clinical Practice. First published: 16 February 2017 https://doi.org/10.1177/0884533616687501
[228] Canani RB, Nocerino R, Frediani T, Lucarelli S, Di Scala C, Varin E, Leone L, Muraro A, Agostoni C. Amino Acid-based Formula in Cow's Milk Allergy: Long-term Effects on Body Growth and Protein Metabolism. J Pediatr Gastroenterol Nutr. 2017 Apr;64(4):632-638. doi: 10.1097/MPG.0000000000001337. PMID: 27437928.

[229] Elango R, Chapman K, Rafii M, Ball RO, Pencharz PB. Determination of the tolerable upper intake level of leucine in acute dietary studies in young men. Am J Clin Nutr. 2012 Oct;96(4):759-67. Epub 2012 Sep 5

[230] 21 CFR Chpt 1 172.320: http://a257.g.akamaitech.net/7/257/2422/10apr20061500/edocket.access.gpo.gov/cfr_2006/aprqtr/pdf/21cfr172.320.pdf Accessed 11-07-14.